SAFETY AND PHARMACOLOGICAL PROFILE OF *PANCHAKKINI CHENDURAM*

The dissertation Submitted by

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CONTENTS

INTRODUCTION

1. INTRODUCTION

Siddha system is the one of the ancient system of medicine in the world. Siddhars are the pioneers of Siddha system of medicines. It includes 32 types of internal and 32 types of external medicines. The siddha drugs have been prepared from herbs, metals, minerals and animal resources. A total number of 4448 diseases are mentioned in siddha text as well as with line of treatment.

The term 'Siddhar' has derived from the word 'Siddhi' which literally means accomplished, achieved or perfected success and so it refers to one who had attained his end in spiritual goal.

The major contributions of Siddhars are classified as follows

- Alchemy
- Medicine
- Meditation
- Knowledge

Back to medicine, Siddhars had an unparalleled knowledge of medicine preparation. The worldly objects are classified under the following three divisions

- Plant kingdom
- Mineral kingdom and
- Animal kingdom

In Siddha, *Chenduram* (calcined red oxide) is one of the greatest higher order medicines. It is a Metallic substance or toxic salts are made into red coloured powder, by the process of either burning or drying them or exposing to the sunlight or keeping them in specialized tubes by adding decoction, liquid of victory (*ceyaneer)*, acid etc..

Chenduram is a very effective medicine even in small dose and it has the longer shelf life even upto 75 years. It can be given with the sustained adjuvant. The outcome of this medicine is faster also has the ability to treat chronic and medically challenging diseases but should be prepared in a proper method.

Chenduram has the energy to postponed the ageing process. So, the energy of *Karpa Chenduram* is much greater than the *Karpa plants.*

The saint *Agasthiyar* and *Yagoppu* have written books on *Chenduram* namely *Agasthiyar Chenduram* 800, *Loga Chenduram* 300 and *Chendura Soothiram* 150 respectively.

Panchakkini Chenduram is one among the mineral formulation mentioned in the text *Anuboga vaidya navaneetham*, vol 1 page 92 Author- Hakkim Abdulla shayabu.

The ingredients of "*Panchakkini chenduram*" are Purified *Arappodi* (Iron), Purified *Kaantham* (Magnetic Oxide of Iron), *Sangu Chunnam* (Conch Shell), Purified *Nellikkai Gandhagam* (Sulphur) and Purified*Navacharam* (Ammonium Chloride). According to the Siddha text, ingredients of *Panchakkini chenduram* is mentioned as therapeutically effective for *Manjal Noi* (Liver disease), *Paandu* (Anaemia), *Neerkattu* (Oliguria).

Panchakkini Chenduram has not been scientifically validated so far and author has selected *Panchakkini chenduram* for the evaluation of safety study, Hepatoprotective activity, Diuretic and Hematinic activity.

AIM AND OBJECTIVES

2. AIM AND OBJECTIVES

AIM

To evaluate the safety and pharmacological aspect of the test drug "*Panchakkini Chenduram"* in animal models.

OBJECTIVES

Panchakkini Chenduram has been evaluated in the following aspects.

1. Collection of evidence in *Siddha* literature.
2. Collection of evidence in mineral and metals.
3. Pharmacognostical study of the test drug.
4. Physicochemical, chemical and phytochemical analysis of test drug.

5. **Toxicity studies**
   - ❖ Acute oral toxicity study of the drug by OECD-423 Guideline.
   - ❖ 28 days Repeated oral toxicity study of the drug by OECD-407 Guideline.
   - ❖ 90 days Repeated oral toxicity study of the drug by OECD-408 Guideline.

6. Pharmacological activities in Wister albino rats

- ❖ Hepatoprotective activity by Paracetamol induced method.
- ❖ Diuretic activity by Lipschitz method.
- ❖ Hematinic activity by Phenyl hydrazine method.

REVIEW OF LITERATURE

MINERALOGICAL ASPECT

3. REVIEW OF LITERATURE

3.1 MINERALOGICAL ASPECT

IRON [2,3,4]

GENERAL PROPERTIES

Name	:	Iron
Empirical formula	:	Feo
Symbol	:	Fe
Appearance	:	lustrous metallic with a tinge

- Iron is an essential mineral that contributes too many important physiologic functions in the body.
- Much of the iron in the body is attached to hemoglobin molecules in red blood cells, thereby delivering oxygen to all of the tissues.
- Extra iron is stored in the liver, bone marrow, spleen and muscles.

VERNACULAR NAMES

Tamil	:	*Irimbu*
Sanskrit	:	Lauha, hyam
Malayalam	:	Basi
Telugu	:	Inumu
Urdu	:	Lohchun

SOURCE

- Widely distributed in both the organic and inorganic kingdoms.
- Found in nearly all rocks, soils, etc, variously combined with oxygen as haematite, with sulphur as iron pyrites.
- Found in the ashes of plants and even the blood (RBC of the blood) of animals and also in the bile, chyle, gastric juice, lymph, milk, pigment of the eye and in the urine.
- The largest iron resources in the world are in China, Russia, Brazil, Canada, Australia and India.

FOOD SOURCES OF IRON

Good sources of iron include,

- Liver
- Meat
- Beans
- Nuts
- Fruits (such as dried apricots)
- Whole grains (such as brown rice) fortified breakfast cereals
- Soybean flour
- Most dark green leafy vegetables (such as watercress, curly kale & spinach)

PHYSICAL PROPERTIES

- Iron is a silvery-white or grayish metal.
- It is ductile and malleable.
- Iron has a very high tensile strength.

Melting point	:	1,536ºC (2,797ºF)
Boiling point	:	3,000ºC (5,400ºF)
Color	:	Iron black, Dark gray, Steel gray
Density	:	7.3-7.9, Average = 7.6
Diaphaneity	:	Opaque
Fracture	:	Hackly – Jagged, torn surfaces, (eg: fractured Metals)
Hardness	:	4-5- Fluorite-Apatite
Luminescence	:	Non-fluorescent
Luster	:	Metallic
Magnetism	:	Naturally strong
Streak	:	Gray

CHEMICAL PROPERTIES

- Iron is a very active metal.
- It readily combines with oxygen in moist air.
- The product of this reaction, iron oxide ($Fe_2 o_3$) is known as rust.
- Iron also reacts with very hot water and steam to produce hydrogen gas.
- It also dissolves in most acids and reacts with many other elements.

ACTION

- Iron improves the quality of blood.
- Iron stimulates the functional activity of all organs of the body and is therefore a valuable general tonic.

BENEFITS AND FUNCTIONS OF IRON

Iron is of tremendous value to the human body. It is involved in the formation of red blood cells and is a transporter of oxygen to every cell, providing us with the necessary burst of energy we all need to help us through our daily tasks.

Iron also assists the memory and the ability to concentrate and helps build resistance to infection, stress and disease.

அயம்

Ayyam (Iron)[14]

வேறு பெயர்கள்

- அகி
- அயசு
- இடி
- இரும்பு
- ஈச செயம்
- கருங்கொல்
- கருப்பி
- கரும்பு
- கருமணல்
- கரும்பொன்
- கயசு
- கிருஷ்ணவையம்
- காலில்நெகிளம்
- ஆதி
- சத்து
- சிரோசரம்
- சிட்டம்
- திரும்பி
- துண்டம்
- பிண்டம்
- பொன்மணல்
- லோகம்
- கருந்தாது
- அயில்
- வாழ்பூமி நாதம்

சுவை

பெரும்பான்மை துவர்ப்பு, சிறுபான்மை புளிப்புக் கைப்பு

வீரியம்

வெப்பம்

செய்கை

- பசியுண்டாக்கி
- உடல் உரமாக்கி
- குருதி பெருக்கி
- உடல் தேற்றி

பொதுக்குணம்

"பாண்டுவெண் குட்டம் பருந்தூல நோய்சோபை
மாண்டிடச்செய் மந்தகா மாலைகுன்மம் பூண்ட
பெருந்தாது நட்டமும்போம் பேதிபசி யுண்டாங்
கருந்தாது நட்டமிடுங் கால்."

தீரும் பிணிகள்

- பித்த பாண்டு
- வெண் குட்டம்
- அதிதூலம் நோய்
- சோபை
- மந்தம்
- காமாலை
- குன்மம்
- சுக்கிலநட்டம்
- கழிச்சல்.
- பசி உண்டாக்கும்.

சுத்தி

இரும்பின் அரப்பொடியை எலுமிச்சம் பழச்சாறு, காடி, நாட்டுக் காட்டாணக்குப் பால் இவை ஒவ்வொன்றிலும் மூன்று நாள் ஊற வைத்துக் கழுவி எடுக்கச் சுத்தியாம்.

அளவு

- உத்தமப் பிரமாணம்-சாமையரிசி அளவு
- மத்திமப் பிரமாணம்-தினை அளவு
- அதமப் பிரமாணம் -நெல்லரிசி

நாள் அளவு

- உத்தமம்-முப்பத்திரண்டு நாள்
- மத்திமம்-பதினெட்டு நாள்
- அதமம் -பன்னிரண்டு நாள்

MAGNETIC OXIDE OF IRON[5, 6]

Chemical formula - Fe_3o_4

Empirical formula - $Fe^{3+}2Fe^{2+}o_4$

- Magnetite is one of the most common oxide minerals and also one of the most common iron minerals. Chemical formula (Fe_3o_4)
- Magnetite is the most magnetic of all the naturally occurring minerals on Earth.
- The chemical IUPAC name is iron (II, III) oxide and the common chemical name is **Ferrous – Ferric oxide**.

SOURCE

Magnetite also occurs in many sedimentary rocks, including banded iron formation.

PHYSICAL PROPERTIES

Cleavage	:	none
Color	:	Grayish black, Iron black
Luster	:	metallic to sub metallic
Magnetism	:	Naturally strong
Streak	:	Black
Hardness	:	5.5-6.5
Diaphaneity	:	Opaque
Specific gravity	:	5.2
Luminescence	:	Non- fluorescent
Density	:	5.1-5.2, average =5.15
Fracture	:	Sub conchoidal-fractures developed in brittle materials Characterized by semi curving surfaces
Curie temperature	:	858k(585ºC,1085ºF)

CHEMICAL PROPERTIES

Magnetite reacts with oxygen to produce hematite and mineral which forms a buffer that can control oxygen fugacity.

USES OF MAGNETITE

- Important ore of iron
- Abrasive
- Toner in electro photography
- Heavy media for specific gravity separations
- Micronutrient in fertilizer, pigment in paints
- An aggregate for high-density concrete

காந்தம்

KAANTHAM (MAGNETIC OXIDE OF IRON)[14]

வேறு பெயர்கள்

- சிவலோகச் சேவகன்
- தரணிக்கு நாதம்
- நவலோகத் துரட்டி
- காயசித்திக்குப் பாத்திர வான்
- சூத அங்குசம்
- முருகன் புராணம்

செய்கை

- குருதிப்பெருக்கி
- பசியுண்டாக்கி
- உடல்தேற்றி
- உடல் உரமாக்கி.

பொதுக்குணம்

"காந்தத்தாற் சோபைகுன்மங் காமிலமே கம்பாண்டு
சேர்ந்ததிரி தோடவெட்டை சீதங்கால் - ஓய்ந்தபசி
பேருதரங் கண்ணோய் பிரமியநீ ராமையும்போம்
ஓரினீறை யாயுளுறும் உன்."

தீரும் நோய்கள்

- ❖ வீக்கம்
- ❖ குன்மம்
- ❖ காமாலை
- ❖ மேகம்
- ❖ பாண்டு
- ❖ முத்தோடம்
- ❖ வெள்ளை வீழல்
- ❖ சீதளம்
- ❖ வாதநோய்
- ❖ மந்தம்
- ❖ மகோதரம்
- ❖ விழிநோய்
- ❖ பிரமியம்
- ❖ நீராமைக்கட்டி முதலியன நீங்கும்.
- ❖ பூரண ஆயுளும் உண்டாம்.

சுத்தி

எலுமிச்சம்பழச்சாறு, புளித்தக் காடி, புளித்த மோர் இவைகளின் முறையே மும்மூன்று நாள் காந்தத்தை ஊறவைத்து வெயிலில் வைத்துக் கழுவி எடுக்கச் சுத்தியாம்.

SULPHUR[4, 7, 8]

- Sulphur is a yellow, non-metallic element with medicinal properties. It has a Distinct "rotten egg" smell, caused by sulphur dioxide gas escaping into the air.
- As a supplement, sulphur is available in two forms- dimethyl sulfoxide (DMSO) and methylsulfonylmethane (MSM). About 15% of DMSO breaks down into MSM in the body.
- Both have been touted as treatments for pain.
- Sulphur occurs in combination with many such as copper, iron etc.
- The chemical properties of sulphur and its compounds including the reaction with mercury, Hg to form a red solid, mercuric sulphide (H_2S).

SYNONYMS

- Sulfur
- Brime stone
- Colloidal sulphur
- Floor of sulphur
- Corosal D & F

VERNACULAR NAMES

Tamil	:	*Gandakam*
Sanskrit	:	Gandhaka
Malayalam	:	Gendagum
Telugu	:	Gandhakam
Hindi	:	Gandak
English	:	Brimstone

AVAILABILITY

Sulphur is procurable in bazaars in different forms myrobalan. Sulphur is used in medicine. It is procurable in all bazaars. It is crystalline in nature.

SULPHUR-S

Atomic number	:	16
Atomic mass	:	$32.6 gmol^{-1}$
Electro negativity	:	2.5

Density	:	2.07g.cm³ at 20 c
Melting point	:	113ºc
Boiling point	:	445ºc
Vanderwaals radius	:	0.127nm
Ionic radiation	:	0.184(-2)nm;0.029(+)
Isotopes	:	5
Electronic shell (Ne)	:	35^{-2} 3p4
Energy of 1 ionisation	:	999.3kj.mol^{-1}
Energy of 2 ionisation	:	2252kj.mol^{-1}
Energy of 3 ionisation	:	3357kj.mol^{-1}
Standard potential	:	-0.51v
Discovered by	:	The ancient

FOOD SOURCES OF SULPHUR

The main sulphur containing foods are red gram, green gram and leafy vegetables. A diet sufficient in protein is generally considered to be adequate in sulphur.

DOSAGE OF SULPHUR

There is no official recommended dietary allowance or dietary reference. Intake for this mineral but as a guideline you need more than 100mg of sulphur per day.

PHYSICO-CHEMICAL PROPERTIES

- Sulphur is a multivalent, non-metal, abundant, tasteless & odorless.
- It is yellow and crystalline solid.
- In nature it is pure elements (or) sulphide minerals.
- The smell of the sulphur compare to rotten egg smell, the presence odour because of hydrogen sulphide (H_2S)
- Sulphur presence naturally near the volcanoes.
- Sulphur presence naturally as massive deposits in Texas & Louisian in U.S.A Canada is the main producer.

It is distributed in the world wide such as,

Canada	:	22%
Erusia	:	11%
Saudhi Arabia	:	5%
Germany & Poland	:	4%
France	:	2%

PHYSICAL PROPERTIES

Symbol	:	S
Formula	:	S_8
System	:	Orthorhombic
Atomic Number	:	16
Colour	:	Elemental Sulphur is a bright yellow crystalline solid
Melting Point	:	115.2C
Hardness	:	½ to 2½
Atomic Mass	:	2.065+0.005u
Density	:	2.07g/cm³

CHEMICAL PROPERTIES

Odour	:	Odourless or Faint, rotten egg if not 100% pure
Molecular Weight	:	256.50
Solubility in Water	:	Insoluble
Boiling Point	:	832F
Purity	:	90% to 100%

HEALTH EFFECTS OF SULPHUR

All living things need Sulphur because it is a part of amino acid. It is absolute dietary requirement, the average person takes in around 900 mg of sulphur /day. Mainly Sulphur is a macro nutrient present in both plants & animals. If a person does not gets enough Sulphur in their diet certain health problems such as itchy, improper development of nails, very unusually the lack of sulphur leads to death.

GENERAL USES OF SULPHUR

Sulphur is commercially important in manufacture of chemical such as sulphuric acid. The chemicals are also used for manufacture of sulfa drugs. In agriculture, the Sulphur is the fore most important crop nutritive element and it is also used as a fertilizer, it is also used to manufacture Poultry feeds. Sulphur is used in medicines only after it is refined well.

ACTION

- Keratolytic Activity
- Antifungal
- AntiBacterial Activity
- Antimicrobial
- Alterative and Tonic

(Sulphur is said to clean the blood and help protect us against toxic build-up.)

MEDICINAL USES

Sulphur is used in scabies. The fumes of burning Sulphur are said to cure gout and rheumatic fever. Theorganic Sulphur reduces the motility and invasion of MDA-MB-231 human breast cancer cells. It is also used as the following purposes in medical uses such as. acne, eczema, ring worm, indigestion, diarrhea, vomiting, belching, hemorrhoids and anal fissures especially for women, insomnia, premenstrual syndrome, headache, dizziness, perspiration, mental tension, lack of memory, depression, impotency, cold, cataract, bronchitis, migraine, fever and conjunctivitis.

கந்தகம்

GANDHAGAM (SULPHUR)[14]

வேறு பெயர்கள்

- பரை வீரியம்
- காரிழையின் நாதம்
- அதீதப்பிரகாசம்
- செல்விவிந்து
- பீசம்
- சக்தி
- செந்தூரத்தாதி
- சத்திபீசம்
- தனம்
- நாதம்
- தேவியுரம்
- நாற்றம்
- பொன்வர்ணி
- பரை நாதம்
- இரச சுரோணிதம்

சுவை

கைப்பு துவர்ப்பு

செய்கை

- பித்தநீரை அதிகப்படுத்தும்
- மலமிளக்கி
- உடல் தேற்றி
- வியர்வை பெருக்கி
- கிருமிநாசினி
- தோல், அசுகங்கள் சளிச் சவ்விலுள்ள கோளங்களின் சுரப்பை அதிகப்படுத்தும்.
- விரேகியில் சிறப்பாகச் செயல்பட்டு சுரப்பை அதிகப்படுத்தும்.

வெளியேற்றம்

- வியர்வை, பால், சிறுநீர் இவற்றின் வழியாக வெளிப்படும்.
- அதிக அளவில் அருந்தப் பேதியை உண்டு பண்ணும்.

பொதுக்குணம்

"நெல்லிக்காய்க் கந்திக்கு நீள்பதினெண் குட்டமந்தம்
வல்லை கவிசைகுன்ம வாயுகண்ணோய் - பொல்லா
விடக்கடிவன் மேகநோய் வீறுசுரம் பேதி
திடக்கிரக ணீபம்போந் தேர்."

தீரும் நோய்கள்

- பதினெண்குட்டம்
- மந்தம்
- கல்லீரல்
- வீக்கம்
- பெருவயிறு வகைகளுள் ஒன்றாகிய கவிசை
- குன்மவாயு
- கண்ணோய்கள் கொடுமையைச் செய்கின்ற விடக்கடிகள்
- நாட்பட்ட மேக நோய்கள்
- வாத சுரம்
- பேதி
- நாட்பட்ட கிரகணி கபம்.

தேரையர் பொருட் பண்பு நூலில்,

தாய் மகவை வளர்ப்பது போல நோய்களின் வெப்பத்தை மாற்றி உடம்பைத் தேற்றிக்குமென்பதை,

"மாதர் மகவை வளர்ப்பதுபோ லேயுடம்பை
யாதரவா கத்தேற்றி யாக்கையினால்-மீதாக"

என்ற வரியால் அறியலாம்.

சுத்தி

கந்தகத்தை ஒரு இரும்புக் கரண்டியிலிட்டுச் சிறிது பசுவெண்ணைய் இட்டு உருக்கிப் பசும்பாலில் சாய்க்கவும். இவ்விதம் முப்பது முறை செய்யக் கந்தகம் சுத்தியாம். ஒவ்வொரு முறையும் புதிய பாலையே உபயோகிக்க வேண்டும்.

அளவு

650மி.கிராம்-1கிராம் (10-30 உளுந்தெடை)

1 முதல் 3வராகனெடை (4.2கிராம்-12.6கிராம்) கொடுக்க மலம் கழியும்.

AMMONIUM CHLORIDE – NAVACHARAM [9]

SYNONYMS

- Sal ammoniac
- Salmiac
- Nushadir salt
- Sal armagnac
- Salt armoniack

PROPERTIES

Chemical fomula	:	NH_4Cl
Molar mass	:	53.49 g mol $^{-1}$
Appearance	:	White solid, hygroscopic
Odour	:	Odorless
Density	:	1.5274g/cm³
Melting point	:	338ºc (640ºF;611k) decomposes,sublimes
Boiling point	:	520ºc (968ºF;793k)
Solubility in water	:	244g/L(-15ºc) 294g/L(0ºc) 383.0g/L(25ºc) 454.4g/L(40ºc) 740.8g/L(100ºc)
Solubility product	:	30.9(395g/L)
Solubility	:	Soluble in liquid ammonia, hydrazine, alcohol, methanol, glycerol slightly Soluble in acetone. Insoluble in diethyl ether, ethyl acetate.
Acidity	:	9.24

ACTION

- Caustic
- Expectorant
- Diuretic
- Diaphoretic
- Antibilious

FOOD

- In several countries, ammonium chloride, under the name sal ammoniac or colloquially salmiak is used as food additive under the E number E510, commonly as a yeast nutrient in bread making.
- It is a feed supplement for cattle and an ingredient in nutritive media for yeast and many microorganism.
- Ammonium chloride is used to spice up dark sweets called salty liquorice (very popular in Nordic countries), in baking to give cookies a very crisp texture and in the liquor salmiakki koskenkorva for flavouring. In India and Pakistan, it is called "No shader" and is used to improve the crispness of snacks such as samosas and jalebi.

MEDICINAL USES

- Ammonium chloride is used as an expectorant in cough medicine. Its expectorant action is caused by irrigative action on the bronchial mucosa, which causes the production of excess respiratory tract fluid, which presumably is easier to cough up.
- Ammonium salts are an irritant to the gastric mucosa and may induce nausea and vomiting.
- Ammonium chloride is used as a systemic acidifying agent in treatment of severe metabolic alkalosis, in oral acid loading test to diagnose distal renal tubular acidosis to maintain the urine at an acid pH in the treatment of some urinary-tract disorders.

நவாச்சாரம்

NAVACHARAM (AMMONIUM CHLORIDE)[14]

வேறு பெயர்கள்

- இஷ்டிகை
- சூளிகை
- சல்லிகை
- படு

செய்கை

- கோழையகற்றி
- வியர்வை பெருக்கி
- சிறுநீர் பெருக்கி
- விரண முண்டாக்கி
- பித்தமகற்றி

இது முக்கியமாக நிண நரம்புகள், மாமிசக் கிரந்திகள் மீது தன் வேகத்தைச் செலுத்தும்.

பொதுக்குணம்

"குன்மம் குடற்சூலை கொல்லும் மகோதரத்தை
வன்மையுறு கல்லடைப்பை மாற்றுங்காண்- சன்மக்
கவிச்சுமுத் தோடங் கனவாத நீக்கும்
நவச்சார மாதே நவில்."

தீரும் பிணிகள்

- வயிற்றுவலி
- குடலில் குத்தல்
- பெருவயிறு
- கல்லடைப்பு
- சருமத்தில் புலால் வாசம்
- திரிதோடம்
- கனவாயு இவைகளை நீக்கும்.

மற்றும் இதை,

- ❖ கல்லீரல் வீக்கம்
- ❖ பிலீக நோய்
- ❖ நீர்க்கோவை இரத்த காசம்
- ❖ முகச்சந்தி
- ❖ சூரியாவர்த்த வாதம்
- ❖ சூதகக்கட்டு
- ❖ கக்கிருமல்
- ❖ முறைக்காய்ச்சல்
- ❖ விடாக் காய்ச்சல் இவைகட்கும் உபயோகிக்கலாம்.

சுத்தி

கோமூத்திரத்தை கரைத்து, வடிகட்டிச் சுண்ட எரித்து வெயிலில் உலர்த்தி எடுக்கச் சுத்தியாம்.

அளவு

2 1/2 குன்றி (325மி.கிராம்) முதல் 7 1/2 குன்றி (975மி.கிராம்) வரையாகும். அதிக அளவில் கொடுக்க பேதியாகும்.

ZOOLOGICAL ASPECT

3.2 ZOOLOGICAL ASPECT

CONCH SHELL[10, 11, 12, 13]

(*SANGU*)

SYNONYM

Xachus Pyrum

Gastropoda

Conch is a common name that is applied to a number of different medium to large-size sea snails or their shells.

SCIENTIFIC	**:**	**CLASSIFICATION**
Kingdom	:	Animalia
Phylum	:	Mollusca
Class	:	Gastropoda
Superorder	:	Caenogastropoda
Order	:	Sorbeoconch
Suborder	:	Hypsogastropoda
Infraorder	:	Littorinimorpha
Superfamily	:	Stromboidea
Family	:	Stromboidea
Genus	:	Strombus

SPECIES

Strombus gigas

Strombus pugilis

VERNACULAR NAME

San & Born	-	Shankha
English	-	Conch; conch shell
Duk	-	Sukk
Geej, Mah,& Canada	-	Shankha
Tamil	-	*Sanka, sangu*
Bengal	-	Sankh
Source	-	Indian ocean coasts

CHARACTERS

A porcelaneous shell of an oblong or conical form, the oblong form is bulged is the middle and tapering at each end. The conical variety is peculiar. The upper portion is like cork screw, twisted and tapering at the end. The base is broad. The interior is hollow. The surface is hard of a dull white colour. The upper surface is highly tuberculated, the under surface shining, very brittle and translucent.

FOOD

The meat of conch is eaten raw in salads or cooked, as in burgers, chowders, fritters, and gumbos. All parts of the conch meat are edible.

HUMAN USES

- The animals inside the shell are eaten either raw, as in salads or cooked as in fritters, chowders, gumbos and burgers.
- In East Asian cuisines, the meat is often cut into thin slices and then steamed or stir-fried.
- Conch meat is also often confused with scintilla, which is more accurately whelk meat.
- Conch shells are sometimes used as decoration, as decorative planters, and in cameo making.

BIOLOGICAL ACTIVITY

- Conch shell is used in treating dyspepsia, digestion impairment, malabsorption syndrome and hepatomegaly.
- *Sankha bhasma* is used for ear ache, ulcer and eye troubles and is indicated internally in case of dyspepsia, gonorrhoea, colic dysentery, jaundice, tymphanitis and flatulence.
- *Sankha bhasma* is also used to treat catarrh, sorethroat, cough and asthma.

ACTIONS

- Nutrient
- Anodyne
- Expectorant
- Carminative
- Digestive
- Astringent

சங்கு

SANGU (CONCH SHELL)[14]

வேறு பெயர்கள்

- நந்து
- கம்பு
- வெள்ளை
- சங்கம்
- சுத்தி
- கோடு
- வண்டு
- தேவதத்தம்
- வளை
- வாரணம்
- இடம்புரி

பொதுக்குணம்

"கசிவா மிரத்த பித்தங் கண்ணோய்க ளேகும்
பசியாறும் வாதம் பறக்கு - மிசிவுடனே
தங்கு முளைவிரணந் தானகலு மேவெள்ளைச்
சங்கமது வுண்டாயிற்றான்."

தீரும் பிணிகள்

- இரத்தபித்தம்
- கண்ணோய்கள்
- வாத மிகுதி
- இசிவு
- முளைக்கட்டி முதலியன நீங்கும்.
- பசி உண்டாம்.

செய்கை

- உடல் உரமாக்கி
- துயரடக்கி
- அகட்டுவாயுவகற்றி
- பசித்தீத்தூண்டி

சுத்தி

சங்கைக் கற்சுண்ணாம்பில் புதைத்துத் தாளித்துக் கழுவி எடுக்கச் சுத்தியாகும்.

3.3 PHARMACEUTICAL ASPECT

Chenduram:

Metallic substances or arsenical compounds are made into red coloured powders by the process of burning, frying or insulating or keeping them in specialized *pudams* by grinding them with decoctions, *ceyaneers* juices etc.

- *Chendurams* which become red by burning; Drugs such as *Miruthyunjagam.*
- *Chendurams* which become red by frying; Drugs such as *Ayaveera Chenduram.*
- *Chendurams* which become red by grinding; Drugs such as *Chandamarutha Chenduram*.
- Majority of *Chendurams* used in day to day practice are like that of *Aya Chenduram*.

***Chenduram* types:**

- *Karruppu chenduram* – Oxidium nigrum
- *Manjal chenduram* – Oxidium flarrum
- *Sivappu chenduram* – Oxidium rubrum
- *Seenaveeravaippu chenduram*
- *Thiriloaga chenduram*- red oxide of three metals
- *Naagakkattu chenduram* – red oxide of consolidated zinc
- *Kaayakarpaaya chenduram*
- *Sidigaiaya chenduram*
- *Ayakattu chenduram*

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1. STANDARD OPERATIVE PROCEDURE[1]

"*Panchakkini chenduram***"** is a *Siddha* formulation which is mentioned in *Siddha* text *Anuboga vaidya navaneetham*, vol 1; page 92 Author- Hakkim Abdulla shayabu.

The Ingredients of *Panchakkini chenduram*,

1. Purified *Arappodi* (Iron)	-	35 gm(1*palam*)
2. Purified *Kaantham* (Magnetic oxide of iron)	-	35 gm(1*palam*)
3. *Sangu Chunnam*(Conch shell)	-	35gm(1*palam*)
4. Purified *Nellikkai Gandhagam* (Sulphur)	-	35 gm(1*palam*)
5. Purified *Navacharam* (Ammonium Chloride)	-	35 gm(1*palam*)

Source of Collection:

The drugs were purchased from reputed country raw drug shop, Paris corner in Chennai.

Identification and Authentication of the drug:

The identity and authenticity of the mineral drugs were confirmed by Dr.M.Suresh Gandhi, Department of Geology, University of Madras, Chennai.

Purification of the ingredients:

1. *Arappodi* (Iron)

The iron powder was immersed in lemon juice,vinegar,latex of country castor (Jatropha curcas) each for 3days. Then it was washed.

2. *Kaantham* (Magnetic oxide of iron)

The magnetic oxide of iron was soaked in lemon juice, sour old rice water fermentation and sour butter milk for three days in each. It was then dried in sun shineand washed. Thus its purified form was obtained.

3. *Gandhagam* (Sulphur)

Sulphur was placed in an iron spoon. A small quantity of cow's butter was added. Then the spoon was heated till the butter melts. These mixtures were immersed in inclined position in cow's milk. This procedure was repeated for 30 times to get purified sulphur. Each time fresh milk was used.

4. *Navacharam* (Ammonium Chloride)

It was dissolved in cow urine and filtered. The filtrate was boiled and insolated to get purified form.

5. *Sangu Chunnam* (Conch shell)

The Conch was buried in lime stone, slaked and taken out after washing to get it purified.

Method of preparation:

Purified *Kaantham* and purified *Arappodi* were ground in a *kalvam* for 1hour, then purified *Nellikkai Gandhagam* was added and ground for 1 hour followed by purified *Navacharam* was added and ground for ½ hour, finally *Sangu Chunnam* was added and ground for 6 hour then collected in a porcelain plate and dried in sunlight for 6 hours.

Next day the mixture was placed in a sunlight from morning 10 am to evening 5.00pm for fifteen days continuously. On 16th day, again it was ground in stone mortar for 6hours and collected in a small mud pot covered with suitable lid, junction of the mud pot and lid was sealed with clay smeared cloth, allowed to dry in sun light then it was subjected into *dhaniyaa pudam* for 21 days. On 22nd day the *pudam* was opened and the *chenduram* was ground well and stored in an air tight container.

Labelling:

Name	:	*Panchakkini Chenduram*
Colour	:	Dark brown colour
Dose	:	3-4 Kundrimani (390-520mg)
Adjuvant	:	*Puthina kudineer*
Date of Preparation	:	06.07.2016
Date of expiry	:	75 years

Indication:

Kaamalai (liver diseases), *Neerkattu* (oliguria), *Paandu* (Anaemia).

Therapeutic administration of drug

Form of medicine	:	*Chenduram*
Route of administration	:	Oral
Dose	:	3-4 *Kundrimani* (390-520mg)
Vehicle	:	*Puthina kudineer*

INGREDIENTS OF THE DRUG

GANDHAGAM

(Before purification)

GANDHAGAM

(After purification)

NAVACHARAM

(Before purification)

NAVACHARAM

(After purification)

KAANTHAM

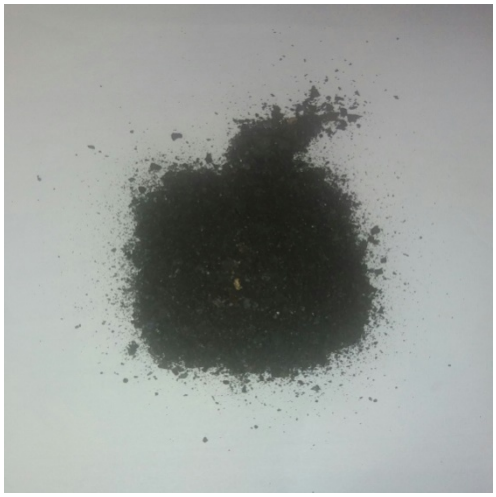
(Before purification)

KAANTHAM

(After purification)

ARAPPODI

(Before purification)



ARAPPODI

(After purification)

SANGU

(Before purification)

SANGU

(After purification)

PANCHAKKINI CHENDURAM

4.2 ANALYTICAL STUDY

ANALYTICAL STUDIES OF *PANCHAKKINI CHENDURAM*

Standardization of the drug brings the validation to be used as a medicine by subjecting the drug into many analysis and determining its quality and effectiveness. Standardization includes many studies such as organoleptic character, physicochemical characteristics studies and determination of phytochemical properties in order to assess the active principles present in the drug. Thus standardization brings the efficacy and potency of the drug.

Standardization of the drug includes:

- Pharmacognostic studies
- Physicochemical analysis
- Chemical analysis
- FT-IR analysis
- XRD analysis
- SEM analysis
- UV analysis

4.2.1 PHARMACOGNOSTIC STUDIES OF *PANCHAKKINI CHENDURAM*

The pharmacognostical study was done at Regional research Institute of Unani Medicine, Royapuram, Chennai. 600013.

4.2.1.1 ORGANOLEPTIC EVALUATION

Colour

The *Panchakkini Chenduram* was taken into watch glasses and placed against white back ground in tube light. It was observed for its colour by naked eye.

Odour

The *Panchakkini Chenduram* was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste

Small amount of *Panchakkini Chenduram* was kept over the tip of the tongue.

4.2.2 PHYSICOCHEMICAL ANALYSIS

Physicochemical studies of the trial drug have been done according to the WHO guidelines.

DETERMINATION OF ASH VALUES

Total Ash

3g of the test drug was accurately weighed and incinerated in a crucible dish at a temperature not exceeding 450° C until it was free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried powder was calculated.

Water Soluble Ash

The total ash was obtained as the above method for preparation of total ash. The ash was boiled with 25ml of water for 5mins. The insoluble ashes were collected using filter paper. It was then washed with hot water and transferred to the silica crucible. It was then ignited for 15minutes at temperature not exceeding 450^{o}C. For determination of weight of the water soluble ash the silica crucible and residue were weighed until constant weight was attained. The weight of the water soluble ash is determined by subtracting the weight of insoluble ash from the weight of total ash.

MATERIALS AND METHODS

Acid insoluble Ash

The total ash was obtained as the above method for preparation of total ash. The ash was boiled for 5 minutes with 25ml 10% Hcl. The insoluble ashes were collected using filter paper and washed with hot water. It was then transferred to the silica crucible and ignited for 15 minutes at temperature not exceeding 450^{o}C. The silica crucible and residue were weighed until constant weight is attained.

DETERMINATION OF EXTRACTIVE VALUE

Alcohol Soluble Extractive Value

3g of test drug powder was weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution was shaken continuously for 6 hours. It was then allowed to stand and soak for 18 hours. The solution was filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C. Then the content was cooled and weighed.

Water soluble Extractive value

3g of test drug powder was weighed and macerated with chloroform and water respectively, at 80°C for 24 hrs. The resulting solution was shaken continuously for 6 hours and allowed to stand and soak for 24hrs then filtered. The solution from both chloroform and water respectively was filtered and evaporated of the filtrate in a flat bottomed shallow dish. It was dried at 105°C then cooled and weighed.

DETERMINATION OF LOSS ON DRYING AT 105°C

The powdered drug was taken and dried in the oven at 105°C to constant weight. The result was noted.

4.2.3 CHEMICAL ANALYSIS OF *PANCHAKKINI CHENDURAM*

The chemical analysis of **"*Panchakkini chenduram*"**was carried out in Bio chemistry lab, National Institute of Siddha.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Physical Appearance of extract	Dark brown in colour	
2.	**Test for Silicate** 500mg of the sample medicine was shaken well with distilled water.	Sparingly soluble	**Presence of Silicate**
3.	**Action of Heat:** 500mg of the sample medicine was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved. No brown fumes evolved.	Absence of Carbonate Absence of Nitrate.
4.	**Flame Test:** 500mg of the sample medicine was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame	Absence of copper
5.	**Ash Test:** A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	Appearance of yellow colour flame	Absense of sodium

Preparation of Extract:

5gm of sample medicine was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation was used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
	I. Test For Acid Radicals		
1.	**Test For Sulphate:** 2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil. ammonium oxalate solution	No cloudy appearance	Absence of sulphate
2.	**Test For Chloride:** 2ml of the above prepared extracts was added with 2ml of dil. HCl is added until the effervescence ceases off.	No cloudy appearance was formed	Absence of Chloride
3	**Test For Phosphate** 2ml of the extract were treated with 2ml of dil. ammonium molybdate solution and 2ml of con.$HN0_3$	Cloudy yellow appearance present	**Presence of phosphate**
4	**Test For Carbonate:** 2ml of the extract was treated with 2ml dil. magnesium sulphate solution	No cloudy appearance.	Absence of carbonate
5.	**Test For Nitrate:** 1gm of the sample medicine was heated with copper turning and concentrated H_2S0_4 and viewed the test tube vertically down.	No Brown gas was evolved	Absence of nitrate
6.	**Test For Sulphide:** 1gm of the sample medicine was treated with 2ml of con. HCl	No rotten egg smelling gas was evolved	Absence of sulphide

7.	**Test For Fluoride & Oxalate:** 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated**.**	No cloudy appearance.	Absence of fluoride and oxalate
8.	**Test For Nitrite:** 3drops of the extract was placed on a filter paper, on that-2 drops of dil. acetic acid and 2 drops of dil.Benzidine solution is placed.	No characteristic changes noted.	Absence of nitrite
9.	**Test For Borate:** 50mg of the sample medicine was made into paste by using dil. Sulphuric acid and alcohol (95%) and introduced into the blue flame.	No appearance bluish green colour.	Absence of borate

II. Test For Basic Radicals			
1.	**Test For Lead:** 2ml of the extract was added with 2ml of dil. potassium iodine solution.	No Yellow precipitate was obtained	Absence of lead
2.	**Test For Copper:** One pinch (25mg) of sample medicine was made into paste with con. HCl in a watch glass and introduced into the non-luminuous part of the flame.	No blue colour precipitate	Absence of copper
3.	**Test For Aluminium:** To the 2ml of extract dil. Sodium hydroxide was added in 5 drops to excess.	No yellow colour appeared	Absence of Aluminium.
4.	**Test For Iron:**To the 2ml of extract add 2ml of dil. Ammonium solution b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added	Red colour appeared	**Presence of Iron**
5.	**Test For Zinc:** To 2ml of the extract dil. Sodium hydroxide solution was added in 5 drops to excess and dil. Ammonium chloride is added.	No White precipitate was formed	Absence of Zinc

6.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil. Ammonium oxalate solution	No Cloudy appearance and white precipitate was formed was is was formed	Absence of calcium
7.	**Test For Magnesium:** To 2ml of extract dil. Sodium hydroxide solution was added in drops to excess.	White precipitate was obtained	**Presence of magnesium**
8.	**Test For Ammonium:** To 2ml of extract 1 ml of Nessler's reagent and excess of dil. Sodium hydroxide solution are added.	No Brown colour appeared	Absence of ammonium
9.	**Test For Potassium:** A pinch (25mg) of sample medicine was treated with 2ml of dil. Sodium nitrite solution and then treated with 2ml of dil. Cobalt nitrate in 30% dil. Glacial acetic acid.	No Yellow precipitate was obtained	Absence of potassium
10.	**Test For Sodium**: 2 pinches (50mg) of the sample medicine is made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved.	Absence of sodium
11.	**Test For Mercury**: 2ml of the extract was treated with 2ml of dil. Sodium hydroxide solution.	No Yellow precipitate was obtained	Absence of Mercury
12.	**Test For Arsenic:** 2ml of the extract was treated with 2ml of dil. Sodium hydroxide solution.	No Brownish red precipitate was obtained	Absence of arsenic
III. Miscellaneous			
1.	**Test For Starch:** 2ml of extract was treated with weak dil.Iodine solution	No Blue colour developed	Absence of starch
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes were noted.	No Brick red colour was developed	Absence of reducing sugar

3.	**Test For The Alkaloids**: a) 2ml of the extract was treated with 2ml of dil. Potassium Iodide solution. b) 2ml of the extract was treated with 2ml of dil. Picric acid. c) 2ml of the extract was treated with 2ml of dil.Phosphotungstic acid.	No red colour developed	Absence of Alkaloid
4.	**Test For Tannic Acid:** 2ml of extract was treated with 2ml of dil. Ferric chloride solution	No Blue-black precipitate was obtained	Absence of Tannic acid
5.	**Test For Unsaturated Compound:** To the 2ml of extract 2ml of dil.Potassium permanganate solution is added.	Potassium permanganate is not decolourised	Absence of unsaturated compound
6.	**Test For Amino Acid:** 2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent is added**.**	No violet colour	Absence of amino Acid
7.	**Test For Type of Compound:** 2ml of the extract was treated with 2 ml of dil. Ferric chloride solution.	No green and red colour No Violet colour developed No Blue colour developed	Absence of quinolepinephrine pyrocatecho anti pyrine, Aliphatic amino acid and meconic acid. Apomorphine salicylate and Resorcinol are absent Morphine, Phenol cresol and hydroquinone are present.

4.2.4 FOURIER TRANSFORM INFRARED (FTIR):

The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of finger print of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is also carried out by using Fourier transform technique.

Description:

The Perkin Elmer Spectrum FTIR instrument consists of globar and mercury vapour lamp as sources, an interferometer chamber comprising of KBr and mylar beam splitters followed by a sample chamber and detector. Entire region of 400-4500 cm-1 is covered by this instrument. The spectrometer works under purged conditions. Solid samples are dispersed in KBr or polyethylene pellets depending on the region of interest. This instrument has a typical resolution of 1.0 cm-1.Signal averaging, signal enhancement, base line correction and other spectral manipulations are possible.

The interference pattern obtained from a two beam interferometer as the path difference between the two beams is altered, when Fourier transformed, gives rise to the spectrum. The transformation of the interferogram into spectrum is carried out mathematically with a dedicated on-line computer.

Model	:	Spectrum 1 FTIR spectrometer
Scan range	:	MIR 450-4500 cm-1
Resolution	:	1.0 cm-1
Sample required	:	50 mg solid or liquid. 58

Sample preparation:

Solid	:	KBr or Nujol mull method
Liquid	:	CsI / T1Br Cells
Gas	:	Gas cells

KBr method :

The sample was grounded using an agate motor and pestle to give a very fine powder. The finely powder sample was mixed with about 100 mg dried potassium bromide salt. The mixture was then pressed under hydraulic press using a die to yield a transparent disc (measure about 13 mm diameter and 0.3 mm in thickness) through which the beam of spectrometer passed.

Applications:

Infrared spectrum is useful in identifying the functional groups like –OH, -CN, -NH2, etc. Also quntitative estimation is possible in certain cases for chemical, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analysed. Blood and food materials can also be analysed[32].

4.2.5 X-RAY DIFFRACTION (POWDER XRD):

PXRD is a compact advanced instrument. When X-rays falls over a crystal, it diffracts in a pattern characteristic to its structure. A diffraction pattern plots Intensity against the angle of detector, 2 . Diffraction occurs when light is scattered by a periodic array with the range of order, producing constructive interference at specific angles. The pattern contains information about the atomic arrangement in crystal. Amorphous materials like glass do not have periodic array with long range order, so they do not produce any significant diffraction pattern.
Sample required: 25gm to be submitted.

Sample preparation:

- Approximately 1gm was kept as a reference, 5gm was taken for sample preparation and the remainder was used for preparation of decalcified, fractioned 2-20μ and less than 2μ samples.
- Sample was disaggregated in waring blenders with 250ml hot distilled water until no lumps of sediment are visible.
- The sample was centrifuged and the wash-water was decanted.

- Then the sample was allowed to dry and disaggregated manually with a mortar and pestle.
- Coarse grained sample was reduced to silt size.
- Then it was placed in mortar and pestle grinders and heat generated grinding done under butanol for 2 hours.
- After grinding, butanol was evaporated under heat lamps.
- The ground sample was treated with trihexylamine acetate
- Then the sample was pressed into sample holder.

Benefits:

It serves a major role in all stage of drug development, testing and production. It is an essential part of analytical research and development, quality control of the active ingredients, excipients and final products. It helps in elucidation of the relevant polymorphic and pseudo-polymorphic forms in pharmaceutical development.

Advantage:

The PXRD analysis of crystalline compounds gives a diffraction pattern consisting of a well defined, narrow, sharp and significany peak while amorphous materials do not give clear peaks rather the pattern has noise signals, smeared peak or it can have some short order bumps.

Powder XRD is used to determine the crystallinity by comparing the integrated intensity of the background pattern to that of the sharp peaks[33].

4.2.6 SCANNING ELECTRON MICROSCOPE (HR-SEM):

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top – down, and with the necessary preparation and sample preparation, cross-section. The Quanta 200 FEG scanning electron microscope (SEM) is a versatile high resolution scanning electron microscope with three modes of operation namely, High vaccum (HV) mode for metallic (electrically conducting) sample,

Low vaccum (LV) modes for insulating, ceramic, polymeric (electrically insulating)

Environment scanning electron microscope (ESEM) for biological samples respectively. Apart from giving the high resolution surface morphological images, the Quanta 200 FEG also has the analytical capabilities such as detecting the presence of elements down to boron on any solid conducting materials through the energy dispersive X-ray spectrometry (EDX) providing crystalline information from the few nanometre depth of the material surface via electron back scattered detection (BSD) system attached with microscope and advanced technological PBS (WDS) for elemental analysis.

Resolution	:	1.2 nm gold particle separation on a carbon substrate
Magnification	:	From a minimum of 12X to greater than 1, 00,000 X
Application	:	To evaluate grain size, particle size distributions, material homogeneity and inter metallic distributions.

Sample required:

Any dimension (Height or Diameter) less than 10 mm. The ideal shape of a sample was that of a button on a shirt. However, the other sizes can also be accommodated only after the discussion with the system operator.

If the sample was not electrically conducting, it will require silver or gold coating. If the sample was a powder, make a normal button size pellet of the sample. If the sample was insulator (or) polymeric (or) electrically non-conducting it needs to be coated with carbon.

Calculation of the particle size:

The horizontal line in the right corner of the micrograph corresponds to micro in length would be given. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the particles was calculated.

Sample preparation:

Sample preparation can be minimal or elaborate for SEM analysis, depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a sample that will fit into the SEM chamber and some accommodation to prevent garge build-up on electrically insulating samples. Most electrically insulating samples are coated with a thin layer of conducting material, commonly carbon, gold, or some other metal or alloy. The choice of material for conductive coatings depends on the data to be acquired. Carbon is most desirable if elemental analysis is a priority, while metal coatings are most effective for high resolution electron imaging applications[34].

4.2.7 ULTRAVIOLET – VISIBLE SPECTROSCOPY

UV spectroscopy is an important tool in analytical chemistry. The other name of UV (Ultra-Violet) spectroscopy is Electronic spectroscopy as it involves the promotion of the electrons from the ground state to the higher energy or excited state.

Introduction to UV Spectroscopy

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Absorption of the ultra-violet radiations results in the excitation of the electrons from the ground state to higher energy state.

Principle of UV Spectroscopy

UV spectroscopy obeys the Beer-Lambert law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

Procedure

Monochromators generally composed of prisms and slits. The most of the spectrophotometers are double beam spectrophotometers. The radiation emitted from the primary source is dispersed with the help of rotating prisms. The various wave lengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wave length to pass through the slits for recording purpose. The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

Uses

Identification of an unknown compound

An unknown compound can be identified with the help of UV spectroscopy. The spectrum of unknown compound is compared with the spectrum of a reference compound and if both the spectrums coincide then it confirms the identification of the unknown substance.

Determination of the purity of a substance

Purity of a substance can also be determined with the help of UV spectroscopy. The absorption of the sample solution is compared with the absorption of the reference solution. The intensity of the absorption can be used for the relative calculation of the purity of the sample substance[35].

TOXICOLOGICAL STUDIES

5. TOXICITY STUDIES

ACUTE ORAL TOXICITY STUDY OF *PANCHAKKINI CHENDURAM* (OECD GUIDELINE – 423)

Introduction:

- The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.
- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
- In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.
- The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

Principle of the Test:

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence

or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

− No further testing is needed

− Dosing of three additional animals, with the same dose

− Dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology:

Selection of Animal Species

The preferred rodent species is the Wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions

The temperature in the experimental animal room should be 22ºC±3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals:

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (150-200gm) were obtained from TANUVAS, Madhavaram, Chennai. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore)

Preparation of animals:

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, *Panchakkini Chenduram.*

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design.

IAEC approved Number: IAEC/XLIX/16/CLBMCP/2016

Test Substance	:	***PANCHAKKINI CHENDURAM***
Animal Source	:	TANUVAS, Madhavaram, Chennai.
Animals	:	Wister Albino Rats (Female-3+3)
Age	:	6-8 weeks
Body Weight	:	150-200gm.
Acclimatization	:	Seven days prior to dosing.
Veterinary examination	:	Prior and at the end of the acclimatization period.
Identification of animals	:	By cage number, animal number and individual marking by using Picric acid.
Number of animals	:	3 Female/group,
Route of administration	:	Oral
Diet	:	Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore

Water	:	Aqua guard portable water in polypropylene bottles.
Housing & Environment	:	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	:	between 22ºC ± 3ºC.
Relative humidity	:	between 30% and 70%,
Air changes	:	10 to 15 per hour and
Dark and light cycle	:	12**:**12 hours.
Duration of the study	:	14 Days

Administration of Doses:

Panchakkini Chenduram was suspended in water and administered to the groups of Wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 5, 50, 300 and 2000 mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then monitered for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Observations:

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane End points Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanly killed. When animals are killed for human reasons or found dead, the time of death was recorded.

5.1 Acute oral toxicity study of *Panchakkini Chenduram*

Dose finding experiment and its behavioral Signs of acute oral Toxicity

Observation done:

SL	Group CONTROL	Observation	SL	Group TEST GROUP	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant Color change	7	Change in skin Color	No significant color Change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity Response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle Gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Behaviour:

The animals will be observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convolusion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, straub, tremor and writhes, diarrhea, leathery, sleep and coma.

Body Weight:

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Mortality:

Animals were observed for mortality throughout the entire period.

5.2 REPEATED DOSE 28-DAY ORAL TOXICITY (407) STUDY OF *PANCHAKKINI CHENDURAM*

Test Substance	:	***PANCHAKKINI CHENDURAM***
Animal Source	:	TANUVAS, Madhavaram, Chennai.
Animals	:	Wistar Albino Rats (Male -24, and Female-24)
Age	**:**	6-8 weeks
Body Weight	**:**	150-200gm.
Acclimatization	**:**	Seven days prior to dose.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid
Diet	**:**	Pellet feed supplied by Sai Meera Foods Pvt Ltd, Bangalore
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	between 22ºC±3ºC.
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.
Duration of the study	**:**	**28 Days.**

Groups	**No of Rats**
Group I Vehicle control (Water)	12 (6male,6 female)
Group II PC- low dose 2.5mg	12 (6male,6 female)
Group III PC- Mid dose 5mg	12 (6male,6female)
Group IV PC- High dose 10mg	12 (6male,6female)

PC – *PANCHAKKINI CHENDURAM*

Methodology

Randomization, Numbering and Grouping of Animals:

48 Wistar Albino Rats (24M + 24F) were selected and divided into 4 groups. Each group consist of 12 animals (Male -6, and Female-6). First group treated as a control and other three group were treated with test drug (low, mid, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They are low dose, mid dose, high dose. X is calculated by multiplying the therapeutic dose (600 mg) and the body surface area of the rat (0.018). Low dose is 2.5mg/kg, Mid dose is 5mg/kg, High dose is 10mg / kg.

Preparation and Administration of Dose:

PANCHAKKINI CHENDURAM suspended in with Puthina kudineer. It was administered to animals at the dose levels of Low, Mid, High. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.

Observations:

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 1, at weekly intervals throughout the course of study.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Necropsy:

All the animals were sacrificed by excessive anesthesia on day 29. Necropsy of all animals was carried out.

TERMINAL STUDIES

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations:

Haematological parameters were determined using Haematology analyzer.

Biochemical Investigations:

Biochemical parameters were determined using auto-analyzer.

Histopathology:

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done

by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin red.

Statistical analysis:

Findings such as body weight changes, water and food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by dunnett's test using a computer software programme – Graph pad version 7. All data were summarized in tabular form, (Table-13 to 19)

5.3 REPEATED DOSE 90-DAY ORAL TOXICITY STUDY OF *PANCHAKKINI CHENDURAM* (OECD GUIDELINE - 408)

Test Substance	:	***PANCHAKKINI CHENDURAM***
Animal Source	:	TANUVAS,Madhavaram, Chennai.
Animals	:	Wistar Albino Rats (Male -40, and Female-40)
Age	**:**	6-8 weeks
Body Weight	**:**	150-200gm.
Acclimatization	**:**	Seven days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using picric acid.
Diet	**:**	Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	between 22ºC $\pm$ 3ºC.
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.
Duration of the study	**:**	90 Days.

Groups	**No of Rats**
Group I Vehicle control	20 (10male, 10female)
Group II PC- low dose 2.5mg	20 (10male, 10female)
Group III PC - Mid dose 5mg	20 (10male, 10female)
Group IV PC - High dose 10 mg	20 (10male,10female)

PC- ***PANCHAKKINI CHENDURAM***

Methodology

Randomization, Numbering and Grouping of Animals:

80 Wistar Albino Rats (40M + 40F) were selected and divided into 4 groups. Each group consists of 20 animals (Male -10 and Female-10). First group treated as a control and other three groups were treated with test drug (low, mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They are low dose, mid dose, high dose. X is calculated from the Therapeutic dose (600mg) and the body surface area of the rat (0.018). Low dose is 2.5mg/kg, Mid dose is 5mg/kg, High dose is 10 mg/kg.

Preparation and Administration of Dose:

PANCHAKKINI CHENDURAM was administered to animals at the dose levels of low, mid and high dose. The control animals were administered vehicle (Puthina kudineer) only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

Observations:

Experimental animals were kept under observation throughout the course of Study for the following:

Body Weight:

Weight of each rat was recorded on day 1,15,30,45,60,75,90, at biweekly intervals throughout the course of study.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Necropsy:

All the animals were sacrificed by excessive anesthesia on day 91. Necropsy of all animals was carried out.

Laboratory Investigations:

Following laboratory investigations were carried out on day 91 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations:

Haematological parameters were determined using Haematology analyzer.

Biochemical Investigations:

Biochemical parameters were determined using auto-analyzer

Histopathology:

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto

technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by dunnett's test using a statistics software Graph Pad version 7. All data were summarized in tabular form (Table 20-25)

PHARMACOLOGICAL STUDIES

6. PHARMOLOGICAL STUDIES

6.1 HEPATOPROTECTIVE ACTIVITY OF *PANCHAKKINI CHENDURAM*

Aim

To study the Hepatoprotective of *Panchakkini Chenduram* in Wistar albino rats by paracetamol induced hepatotoxicity method.

Selection of animals:

Healthy either sex of Wistar albino rats (150-200g) were used for this study with the approval of the institutional animal ethics committee and obtained from the animals laboratory.

IAEC approved Number: IAEC/XLIX/16/CLBMCP/2016

The animals kept in plastic cages and maintained at22ºC ± 30ºC. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hours throughout the studies. Rats were allowed an acclimatization period of 7days before actual experiments. The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Experimental design[15]

The paracetamol was diluted with sucrose solution (40% w/v). (1:1) before administration. The animals were divided into 5 groups of 6 each. The animals were then subjected to either one of the following treatments for 9 days.

Group 1 : Distilled water (1ml/kg, po)
Group 2 : Distilled water for 9 days + Paracetamol (1g/kg, po) on ninth day
Group 3 : Silymarin (100mg/ kg, po) for 9 days + Paracetamol (1g/kg, po) on ninth day
Group 4 : PC (5 mg/kg/day, po) for 9 days+ Paracetamol (1g/kg, po) on ninth day
Group 5 : PC (10mg/kg/day, po) for 9 days+ Paracetamol (1g/kg, po) on ninth day

PC -*PANCHAKKINI CHENDURAM*

Food was withdrawn 12hr before paracetamol administration to enhance the acute liver damage in animals of group 2, 3, 4 and 5. The animals were sacrificed 48hr after the administration of paracetamol. The blood samples were collected.

Blood sample collection and analysis

Blood samples for haematological analysis were collected from all the rats through the retro-orbital venous plexus under ether-induced anaesthesia, into heparinized tubes while the sample for serum biochemistry was collected into plain tubes. From the blood samples collected, packed cell volume (PCV) was determined by micro haematocrit method, haemoglobin concentration(Hb) by cyanmethaemoglobin method while the red blood cells (RBC) and white blood cells (WBC) were counted using haemocytometer. Means corpuscular volume (MCV), means corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)were calculated from the values of PCV, Hb, and RBC count as described by jain ,(1986)[16.] Erythrocyte osmotic fragility were determined according to the method described by oyewale,(1992)[17]by diluting 0.02ml of blood in test tubes containing 0-0.9% NaCl in phosphate buffer at pHof 7.4. The tubes were gently mixed and incubated at room temperature (29ºC) for 30 minutes, then centrifuged at 3500rev/min for 10 minutes. The supernatant were decanted and the optical density determined at 540nm using SM22PC spectrophotometer (surgienfield Instruments, England). Haemolysis in each tube were expressed as a percentage, taking the tube with the highest heamolysis (i.e. Distilled water with 0.0%NaCl)as 100%

Serum biochemistry

Whole blood were separated with high macro-centrifuge at 3,500 rpm for 10 minutes and serum were separated by Pasteur pipette for analysis of the following biochemistry assays; Alkaline phosphate (ALP) as described by Tietz and Shuey (1986)[19,] aspartate aminotransferase(AST), (Bergmeyer et al., 1985)[18], alanine aminotransferase (ALT)(Klauke et al., 1983)[20] albumin (varely , 1994)[21] and total protein (Keller, 1984)[22.]

All results were reported as mean $\pm$ SEM. they were further analyzed using one way analysis of variants (ANOVA) followed by Tukey's multiple comparison test.

6.2 DIURETIC ACTIVITY (LIPSCHITZ TEST)

Aim

To study the Diuretic activity of *Panchakkini Chenduram* in Wistar albino rats by Lipschitz test induced method.

Selection of animals:

Healthy either sex of Wistar albino rats (150-200g) were used for this study with the approval of the institutional animal ethics committee and obtained from the animals laboratory.

IAEC approved Number: IAEC/XLIX/16/CLBMCP/2016

The animals kept in plastic cages and maintained at 22ºC ±30ºC. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animals house they also maintained with alternative dark-light cycle of 12hours throughout the studies. Rats were allowed an acclimatization period of 7days before actual experiments. The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Study design:[23, 24]

Wistar rats were divided into five groups, consisting six rats for each group. Group (I) served as normal control (Vehicle) which received normal Saline water (10 ml/kg orally) only. Group II received as furosemide orally dose of (10mg/kg, p.o), Groups (III) to (V) received *Panchakkini Chenduram* respectively at dose of 2.5mg/kg, 5mg/kg and 10mg/kg respectively. Immediately after the extract treatment, all the rats are hydrated with saline (15 ml/kg) and placed in a metabolic cages. A total volume of urine collected for 6 hours was measured at the end. During this period no food and water were made available to animals. Various parameters like total urine volume and concentration of sodium, potassium and chloride in the urine were measured and estimated respectively.

Procedure

The method of Lipschitz *et al*. was employed for the assessment of diuretic activity. All the animals were hydrated with double distilled water. Food and water were withdrawn 8hours before the administration of drug. Immediately after dosing, all the animals were placed individually in metabolic cages and urine passed by the animals over a period of 24hours was collected in a conical flask. Total urine, output, electrolyte, pH was determined.

Estimation of urine output

Metabolic cage is designed with a stainless steel circular frame. The upper portion is covered with a lid, provided with a wire mesh bottom and a funnel for collecting the urine. Stainless steel sieves were placed in the funnel to retain the faeces, allowing only urine to flow down for collection and measurement. The whole structure is fixed to a metal frame, which keeps the frame in upright position. Conical flask is kept to collect the urine, at the bottom exit of the funnel for a period of 24h.Urine volume is expressed as ml/kg. The room temperature is maintained at 27-29°c.

pH

A calibrated pH mater (model: WTW – series pH – 720) was used to estimate pH of the fresh urine samples.[25]

Computation of diuretic index, Lipschitz value and Na+ /K+ ratio[25]

The following equations were used to compute these parameters.

Diuretic index = (UVt/UVc) ……. (1)

Lipschitz value = (UVt/UVr) ...……. (2)

Na+ /K+ ratio = (UNa+ /UK+) …...……..… (3)

Where UVt = mean urine volume of test group, UVc = mean urine volume of control group, UVr = mean urine volume of reference group, UNa+ = concentration of Na+ in urine of a group, and UK+ = concentration of K+ in urine of a group.

Statistical analysis[25]

For determining the statistical significance, standard deviation, standard error mean and Dunnett's test 1% level significance was employed.

6.3 HEMATINIC ACTIVITY OF *PANCHAKKINI CHENDURAM*

Aim

To study the Hematinic activity of *Panchakkini Chenduram* in Wistar albino rats by Phenyl Hydrazine induced method.

Selection of animals:

Healthy either sex of Wistar albino rats (150-200g) were used for this study with the approval of the institutional animal ethics committee and obtained from the animals laboratory.

IAEC approved Number: IAEC/XLIX/16/CLBMCP/2016

The animals kept in plastic cages and maintained at22ºC ± 30ºC. All the rats were housed individually with free access to food , water and libitum. They were feed with standard diet and kept in well ventilated animals house they also maintained with alternative dark-light cycle of 12hours throughout the studies. Rats were allowed an acclimatization period of 7days before actual experiments. The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animals experiment were performed with accordance legislation on welfare.

Experimental design:[26]

Anemia was induced by intraperioneal injection of phenylhydrazine at 40 mg/kg for 2days (d), as described by A boudoulatif et al. (2008).

Following the injection, rats were divided in four groups of six rats each.

Group I - The control received distillated water

Group II - received phenylhydrazine only (anemic group)

Group III - received the Panchakkini Chenduram at dose of10mg /kg /day

Group IV - received standard as hematinic syrup (Dexorange, Franco –Indian pharmaceuticals pvt. Limited, Mumbai) at 0.68ml/kg/day respectively.

The vehicle, the trial medicine and standard were administered from day 2 to day 15 after phenylhydrazine administration.

Collection of blood sample:

At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. Blood samples were collected from the caudal vein into a micro centrifuge tube containing 50nM ethylenediamine tetra acetic acid (EDTA) for the determinations of hematological profile. The blood was collected without EDTA to other test tubes. The blood was allowed to clot by standing at room temperature for 30 minute and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000rpm for 10minutes, and then the serum (supernatant) was isolated and store at refrigerated until required for analysis.

Biochemical estimations

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978)[27]. Reduced glutathione was estimated by method of Moron et al., (1979)[28]. Haemoglobin was estimated by Cyanmethaemoglobin method (Dacie and Lewis, 1968)[29] (Beacon Diagnostic Kit). The serum GOT was estimated by the method of Reitman and Frankel (1957)[30] RBC, Platelet and WBC counted by the method of Ochei and Kolhatkar, (2000)[31]. PCV counted by the method of Ochei and Kolhatkar, (2000)[31].

Statistical Analysis:

Values were expressed as mean ±SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 was used and $p< 0.01$ and $p< 0.001$ were considered to be significant.

RESULTS

7. RESULTS OF *PANCHAKKINI CHENDURAM*

Many studies have been carried out to bring the efficacy and potency of the drug *Panchakkini Chenduram.* The study includes literary collections, organoleptic character, physicochemical and phytochemical analysis, toxicological study and pharmacological study. The drug *Panchakkini Chenduram* has been selected from the text *"Anuboga vaidya navaneetham, vol 1"***.**

- *Gunapadam* review brings the effectiveness of the drug in the management of Liver Diseases, Oliguria, and Anaemia.
- The pharmacological review explains about the Evaluation of Hepatoprotective, Diuretic and Hematinic.

STANDARDIZATION OF THE TEST DRUG

Standardization of the drug is more essential to derive the efficacy, potency of the drug by analysing it by various studies. Following are the results of physicochemical and phytochemical analysis. Physical characterization and estimation of basic and acidic radicals have been done and tabulated.

Toxicological results of the drug and pharmacological activity of the drug were derived. Its result has been tabulated and interpretation was made below. Thus it is to give a complete justification to bring the effectiveness of the trial drug *Panchakkini Chenduram*.

ORGANOLEPTIC CHARACTER

The following characters have been noted in *Panchakkini Chenduram*.

Table: 1. ORGANOLEPTIC EVALUATION

Colour	Dark Brown
Odour	Unpleasant
Taste	Mild Astringent
Texture	Fine powder

PHYSICOCHEMICAL ANALYSIS

Table: 2. Determination of Ash Values

Percentage of Total Ash, Acid insoluble ash and Water Soluble ash values

Parameter	Percentage%
Total Ash Value	70.56%
Acid insoluble Ash	30.97%
Water Soluble ash	26.76%

Interpretation for ash values

Ash:

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug.

Total ash:

The Total ash value of the drug is 70.56% for *Panchakkini Chenduram.*

Acid insoluble ash:

The acid insoluble ash value of the drug is 30.97% for *Panchakkini Chenduram.*

Water Soluble ash:

The Water Soluble ash value of the drug is 26.76% for *Panchakkini Chenduram.*

Table:3. Determination of Extractive Values

Percentage of Alcohol Soluble and Alcohol Successive Soluble

Parameter	Percentage%
Alcohol Soluble	9.84%
Alcohol Successive Soluble	26.45%

Interpretation for Extractive values

Alcohol Soluble:

The Alcohol Soluble value of the drug is 9.84% for *Panchakkini Chenduram.*

Alcohol Successive Soluble:

The Alcohol Successive Soluble value of the drug is 26.45% for *Panchakkini Chenduram.*

Table:4. Determination of pH values

pH values

Parameter	Values
pH	8.2

Interpretation for pH values:

The pH value of the drug is 8.2 for *Panchakkini Chenduram.*

Table:5. Loss on Drying

Percentage Loss in weight on drying

Parameter	Percentage%
Loss on drying	2.353%

INTERPRETATION

- The total of volatile content and moisture present in the drug was established in loss on drying.
- Moisture content of the drug reveals the stability and its shelf-life.
- High moisture content can adversely affect the active ingredient of the drug.
- Thus low moisture content could get maximum stability and better shelf life.
- The loss on drying value of the drug is 2.353% for *Panchakkini Chenduram.*

Chemical analysis

The Chemical analysis shows the presence of **Silicate, Phosphate, Iron and Magnesium** in *Panchakkini Chenduram.*

Table: 6. Chemical Analysis of *Panchakkini Chenduram*

S.NO	Parameters	Results
1.	Silicate	**Present**
2.	Sulphate	Absent
3.	Chloride	Absent
4.	Phosphate	**Present**
5.	Carbonate	Absent
6.	Nitrate	Absent
7.	Sulphide	Absent
8.	Oxalate	Absent
9.	Nitrite	Absent
10.	Borate	Absent
11.	Lead	Absent
12.	Copper	Absent
13.	Aluminium	Absent

Interpretation

The acidic radicals test shows the presence of **Silicate and Phosphate**

Table: 7.Chemical Analysis of *Panchakkini Chenduram*

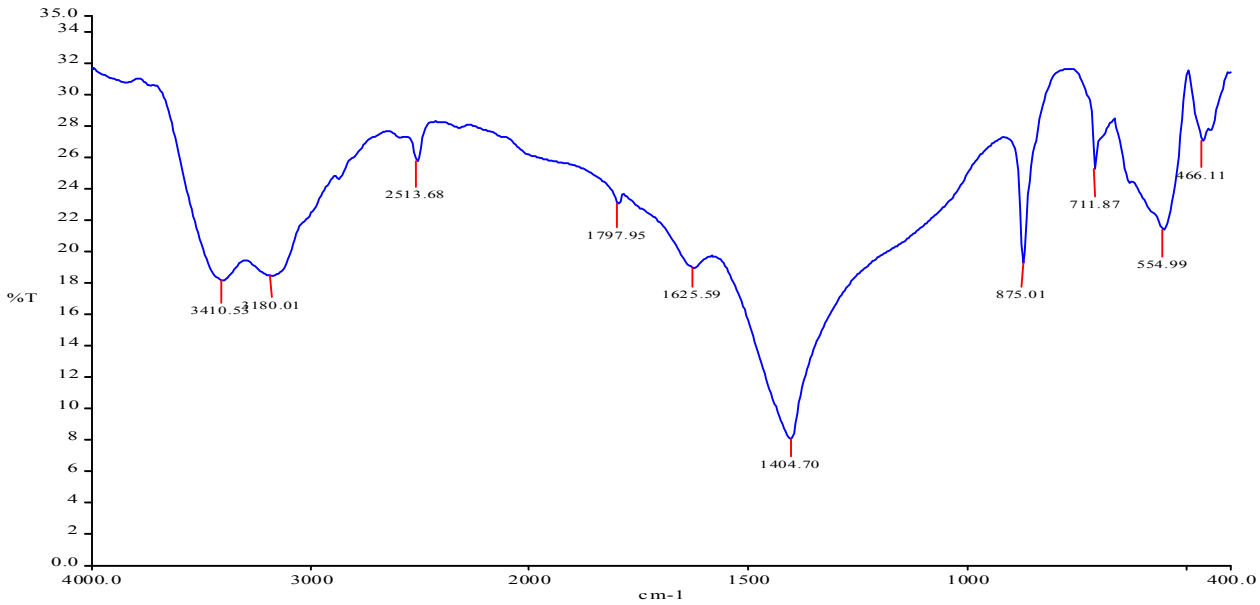
S.NO	Parameters	Results
14.	Iron	**Present**
15.	Zinc	Absent
16.	Calcium	Absent
17.	Magnesium	**Present**
18.	Ammonium	Absent
19.	Potassium	Absent
20.	Sodium	Absent
21.	Mercury	Absent
22.	Arsenic	Absent
23.	Starch	Absent
24.	Reducing sugar	Absent
25.	Alkaloids	Absent
26.	Tannic acid	Absent

Interpretation

The basic radical test shows the presence of **Iron, Magnesium** and absence of heavy metals such as lead, arsenic and mercury.

ANALYTICAL STUDY

FT-IR SPECTRUM



SR No 17-03-X-2530-160317.pk

SR No 17-03-X-2530-160317.005 3601 4000.00 400.00 8.08 31.66 4.00 %T 16 0.50

REF 4000 31.65 2000 26.13 600
3410.53 18.16 3180.01 18.45 2513.68 25.74 1797.95 23.04 1625.59 18.96
1404.70 8.08 875.01 19.23 711.87 25.22 554.99 21.37 466.11 27.05

Result Analysis Interpretation

- Strong intense peak at 466.11 cm-1 may be due to Fe- S stretching indicates the presence of Fe-S group
- Wide intensity peal at 554.9 cm-1 may be due to the presence of S-S organic sulfide group
- IR absorbance peal appears at 711.87 and 875.01 due to the –S stretching
- Sharp intense peak at 1404.70cm-1 is due to presence of C-O stretching
- Weak absorbance at 3410.53cm-1 may be due to NH primary amine stretching
- IR absorption peak at 1625.59 cm-1 due to NH_2 scissoring
- IR absorption peak at 2513.68 and 3180.01cm-1 due to presence of O-H functional group stretching.

XRD ANALUSIS STUDY

- Less intense peak appears at 1797.95 cm-1 due to presence of C=O stret

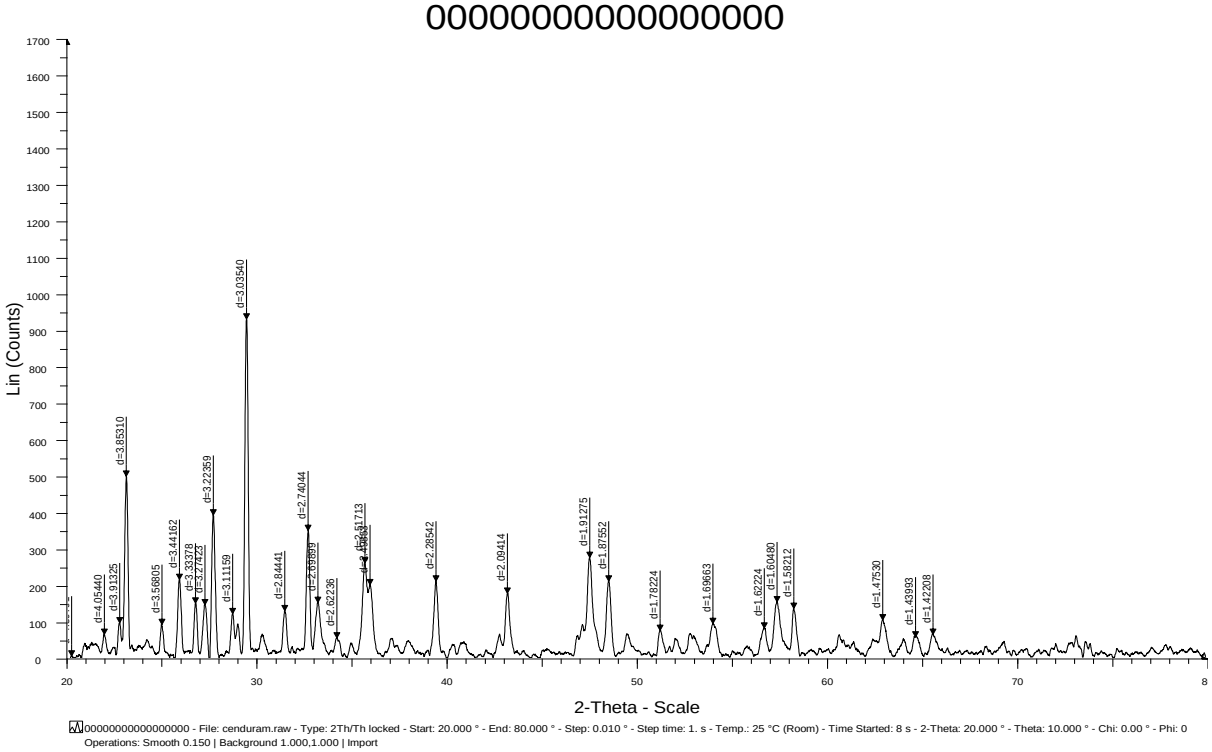


Table: 8 Crystalline:

Sample Name	Left Angle	Right Angle	Left Int.	Right Int.	**Obs. Max**	d (Obs. Max)	Max Int.	Net Height	**FWHM**	Chord Mid.	I. Breadth	Gravity C.	d (Gravity C.)	Raw Area	Net Area
	2-Theta °	2-Theta °	Cps	Cps	**2-Theta °**	Angstrom	Cps	Cps	**2-Theta °**	2-Theta °	2-Theta °	2-Theta °	Angstrom	Cps x 2-Theta °	Cps x 2-Theta °
00000000000000000	29.270	29.530	240	240	**29.402**	3.03541	932	692	**0.164**	29.401	0.159	29.401	3.03547	172.7	110.3

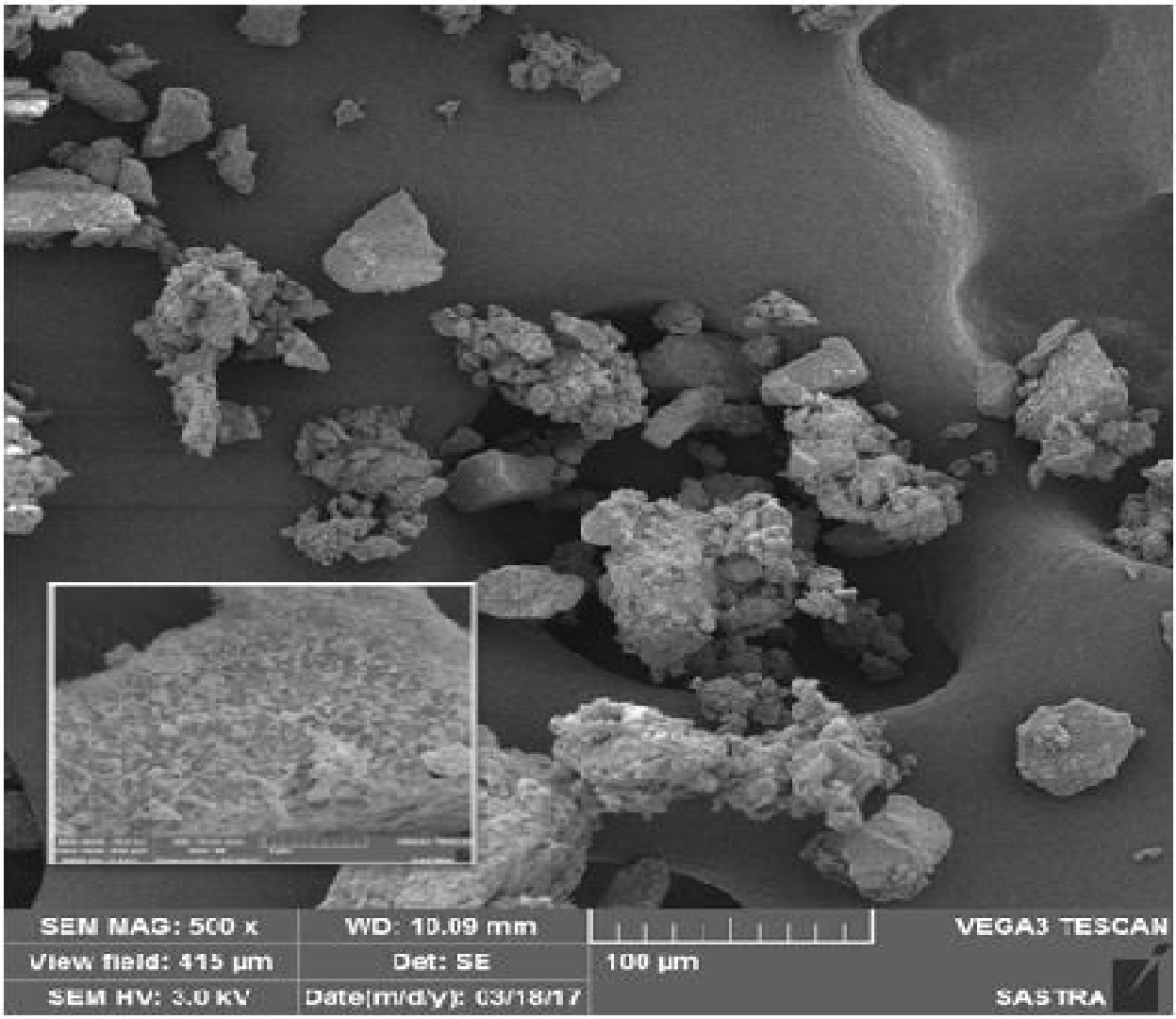
Result Analysis of XRD pattern of *Panchakkini Chenduram*

- The X-ray diffraction pattern of the of the sample *Chenduram* reveals the presence of major peak with 2- Theta value of 29.402 which exactly matches with the following ICDD's (International Centre for Diffraction Data) 65-2567, 07-0007,89-5892 and 080247.
- ICDD 65-2567 corresponds to the crystalline pattern of Iron Sulfide (FeS_2)

- ICDD 07-0007 corresponds to the crystalline pattern of Ammonium Chloride (NH_4Cl)
- ICDD 89-5892 corresponds to the crystalline pattern of Iron Oxide (Fe_2O_3)
- ICDD 08-0247 corresponds to the crystalline pattern of Sulfur (Fe_2O_3)
- Major peaks observed in test sample *Chenduram* with 2-theta values of 29.402 and their corresponding intensities were 933.
- The major peak observed in the reference matching material Fes2 was 33.22 with the intensity value of 999 and in NH_4Cl it was 32.65 with intensity value of 100.
- Similarly the major peak observed in the reference matching material Fe_2O_3 was 35.65 with the intensity value of 999 and in sulfur it was 23.08 with intensity value of 100.
- The XRD pattern of the test sample (*Chenduram*) matches with the following reference materials such as Iron Sulfide, Ammonium Chloride, Iron Oxide and sulfur which justifies the presence of stable and purified nature of above mentioned compounds in the form ingredients in the test sample.
- From the result of the present XRD analysis it was concluded that the elemental composition of *Chenduram* comprises of the mixture of Iron Sulfide, Ammonium Chloride, Iron Oxide and sulfur.

SEM ANALYSIS

Figure 1 : SEM image of *Panchakki Chenduram* – Cluster View



SEM image of *Panchakki Chenduram* – Categorised View

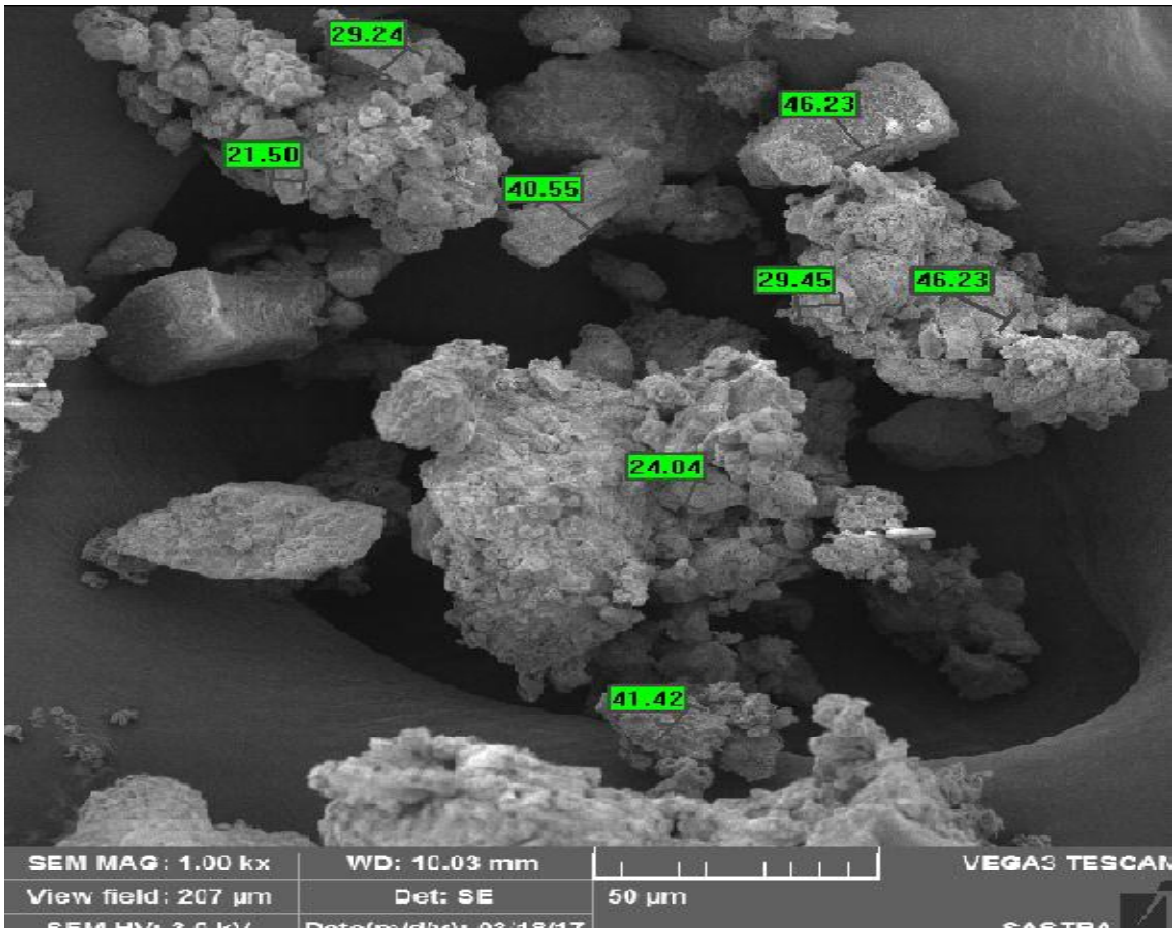


Figure 1A : Particle Size ranges from 21.5 to 46.23 µm Average particle size

Mean	:	34.83
Std Deviation	:	9.931
Std Error	:	3.511

SEM image of *Panchakkini Chenduram*-Categorised View

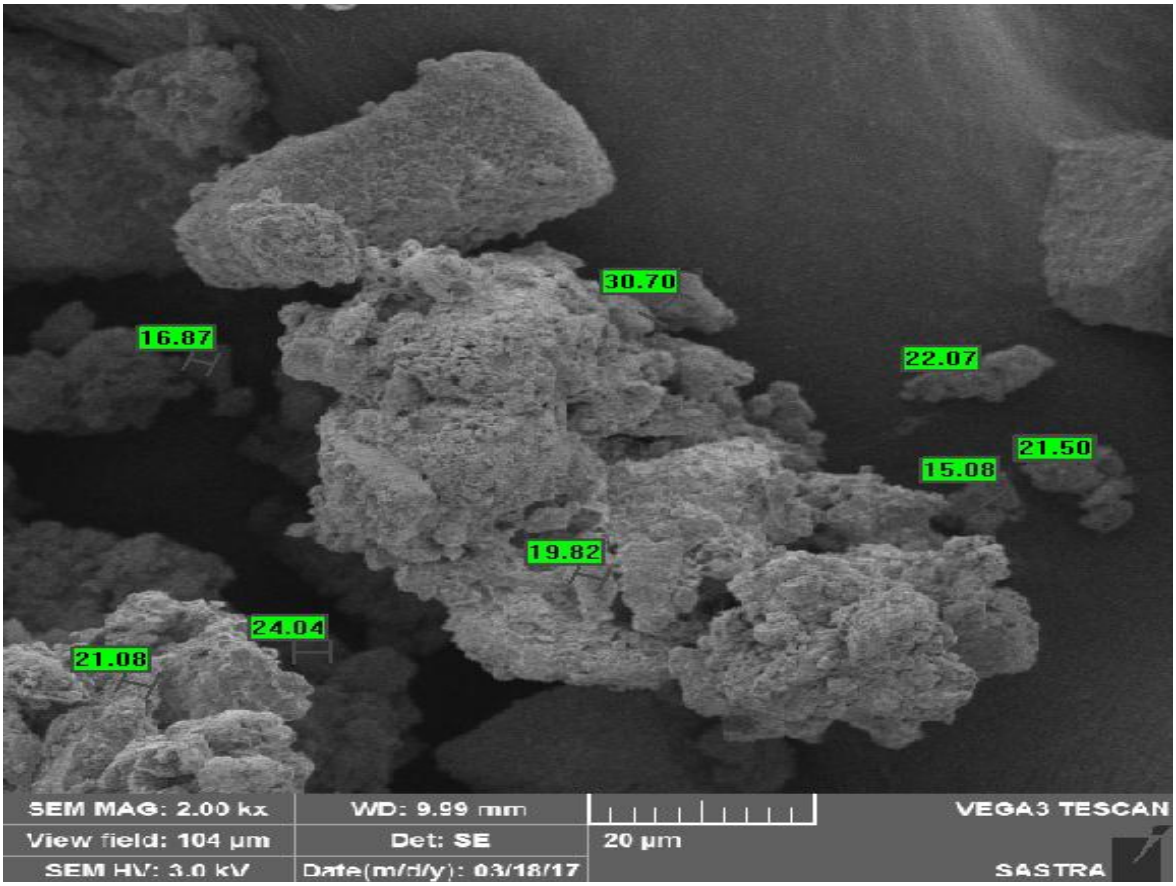
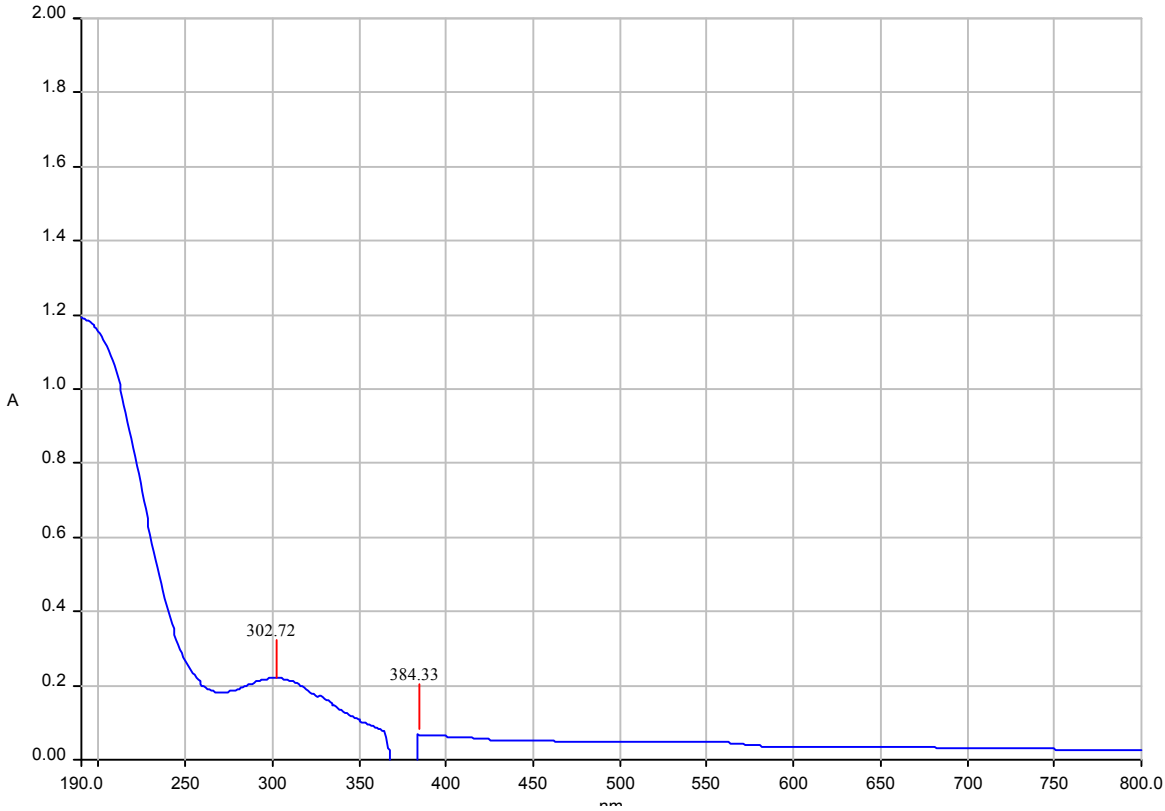


Figure 1B: Particle Size ranges from 15.08 to 30.7 µm

Average Particle size

Mean	:	21. 39
Std. Deviation	:	4.745
Std. Error	:	1.678

UV INTERPRETATION REPORT OF *PANCHAKKINI CHENDURAM*



Report

- The λ max value of the sample *Panchakkini Chenduram* projects intense absorbance at 302.72 nm and 384.33 nm
- Excitation maximum of sample at 302.72nm is a characteristic feature of presence of sulfide group
- Absorbance at 384.33 may be due to the presence of Iron oxide

ACUTE TOXICITY STUDY

Results:

All data were summarized in tabular form,(Table-9-12) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test, description of toxic symptoms, weight changes, food and water in take No of animals in each group:3

Table: 9

(Observational study Results)

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	2000mg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1.Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhea 18. Writhing 19. Respiration 20. Mortality.

(+) indicates the presence

(-) indicates the absence

Interpretation

The acute toxicity result shows no mortality rate up to dose level of 2000mg/kg. It showed changes in alertness, grooming, touch response and grip strength. The normal behavioural changes were observed in first hours and no mortality were reported after 14 days observation. Hence the test drug *Panchakkini Chenduram* is a safe up to the dose of level of 2000mg/kg in oral administration.

Table: 10 (Body weight Observation) Body weight (kg/day) of Wistar albino rats group exposed to *Panchakkini Chenduram.*

DOSE	DAYS		
	1	7	14
CONTROL	320.2±42.30	322.4 ± 60.10	323.6 ±52.10
HIGH DOSE	302.4± 1.21	302 ± 2.04	304.2 ± 2.10
P value (p)*	NS	NS	NS

Table: 11 Water intake (ml/day) of Wistar albino rats group exposed to *Panchakkini Chenduram.*

DOSE	DAYS		
	1	7	14
CONTROL	58 ± 1.02	58±9.20	59.4±1.04
HIGH DOSE	59.4±2.20	59.8±3.40	59.9±6.24
P value (p)*	NS	NS	NS

N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)

Table 12: Food intake (gm/day) of Wistar albino rats group exposed to *Panchakkini Chenduram*

DOSE	DAYS		
	1	7	14
CONTROL	61.04±2.62	62.2±4.76	64.3±6.26
High DOSE	69.4±4.23	70.4±6.22	71.6±4.18

RESULTS

Repeated Dose 28- day oral toxic study of *Panchakkini Chenduram* Body weight of Wistar albino rats group exposed to *Panchakkini Chenduram*

Table 13

DOSE	DAYS				
	1	7	14	21	28
CONTROL	235.2±18.46	236.5 ± 35.10	236.6 ± 45.60	238.7± 56.16	238.4 ± 66.15
LOW DOSE	248.2 ± 65.24	250.7 ± 66.28	254.6± 55.34	256 ±56.34	256.8± 35.36
MID DOSE	252.4± 18.34	253.3 ± 16.24	253.4 ± 14.12	255.2 ± 15.20	256.4 ± 54.10
HIGH DOSE	261.6± 62.24	261.4±42.22	262.4 ± 52.24	263 ± 54.28	264 ± 74.60
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 14: Water intake (ml/day) of Wistar albino rats group exposed to *Panchakkini Chenduram*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	60.1 ± 8.72	60±1.52	60.2±1.40	61±1.32	61.4±1.62
LOW DOSE	65.1±1.21	65.6±4.22	66.6±1.02	65.2±2.06	66.4±1.20
MID DOSE	62.1±1.02	62.3±1.21	62.1±2.62	63.4±4.32	63.4±1.64
HIGH DOSE	64.1±1.81	64.2±1.32	64.4±1.14	64.6±1.62	65.8±2.02
P value (p)*	NS	NS	NS	NS	NS

N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 15: Food intake (gm/day) of Wistar albino rats group exposed to *Panchakkini Chenduram*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	34±4.14	34.2±6.12	34.3±2.18	34.2±1.14	34±5.62
LOW DOSE	36.3±1.64	36.3±1.51	36.2±1.51	36.5±1.62	36.5±1.22
MID DOSE	34.1±2.12	34.2±3.50	34.2±2.14	34.2±2.16	35.2±1.64
HIGH DOSE	32.4±1.62	32.1±1.64	32.6±2.36	32.6±1.20	36.4±2.32
P value (p)*	NS	NS	NS	NS	NS

N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

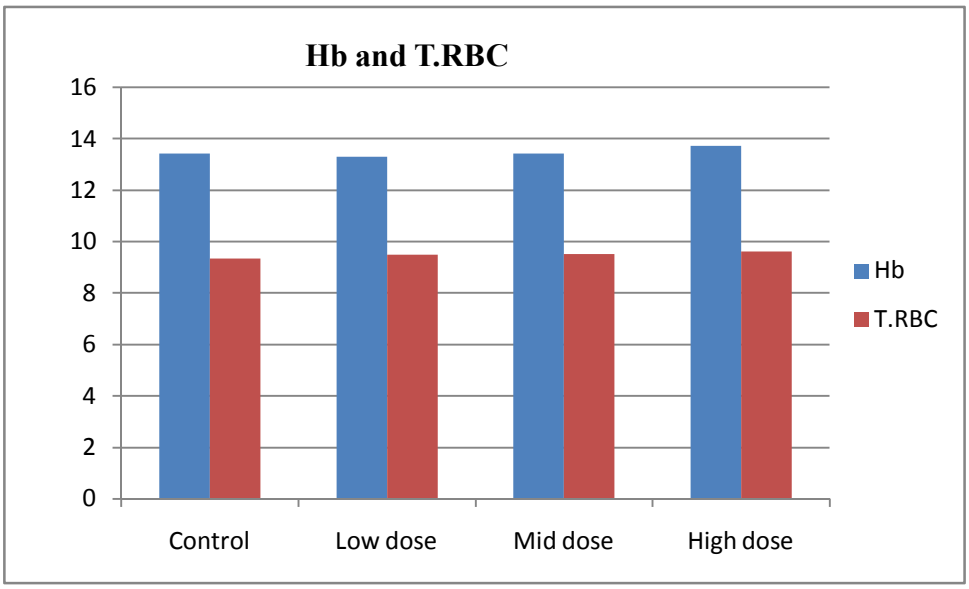
Table 16: Haematological parameters of Wistar albino rats group exposed to *Panchakkini Chenduram*

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin(g/dl)	13.4±0.71	13.30±0.14	13.4±0.13	13.72±0.13	N.S
Total WBC ($\times 10^3$ l)	9.41±0.22	9.32±0.22	9.34±0.22	9.30±1.10	N.S
Platelets cells10^3/µl	900.17±3.18	902.11±4.62	902.11±2.20	902.22±2.64	N.S
Total RBC 10^6/µl	9.32±0.11	9.47±0.33	9.50±0.64	9.60±0.46	N.S
Neutrophils (%)	12.13±0.60	12.02±0.52	12.11±1.42	12.02±2.71	N.S
lymphocyte (%)	82.10±1.26	82.12±1.42	83.10±2.44	83.20±2.54	N.S
Monocyte (%)	1.1±0.03	1.1±0.01	1.2±0.04	1.1±0.03	N.S
Eosinophil (%)	0.8±0.03	0.8±0.04	0.9±0.05	0.9±0.08	N.S
PCV%	48.10±0.2	48.62±5.30	48.8±4.70	48.4±.71	N.S
MCHC g/Dl	36.5±1.61	36.2±1.51	36.8±1.30	36.13±1.60	N.S
MCV fL(µm^3)	58.2±2.02	58.2±1.80	58.7±1.10	59.7±1.30	N.S

N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

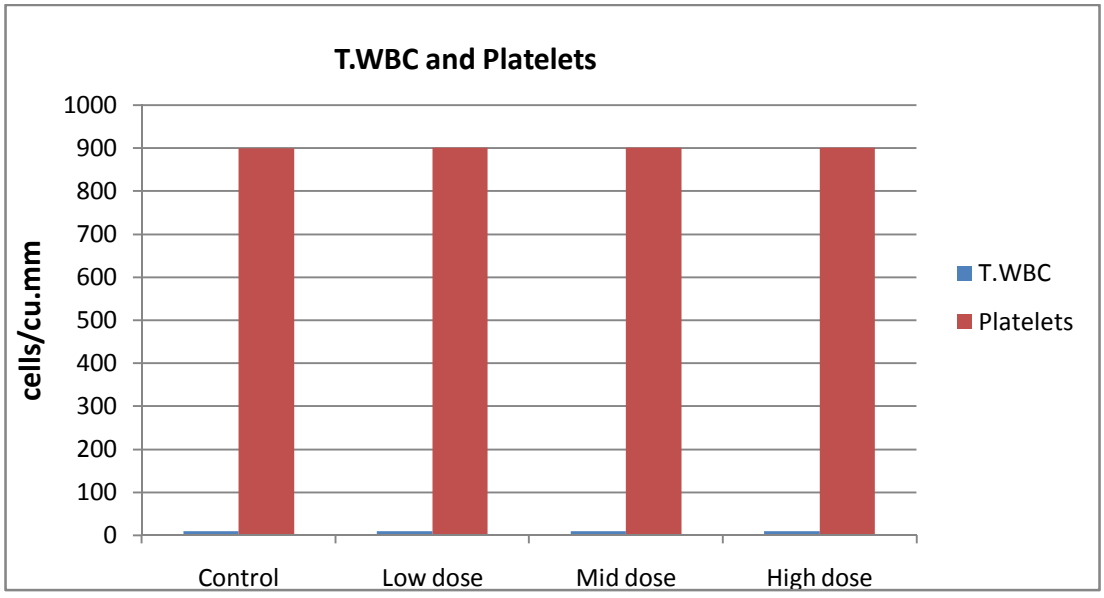
The mean value of Hb% and T.RBC of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart- 1



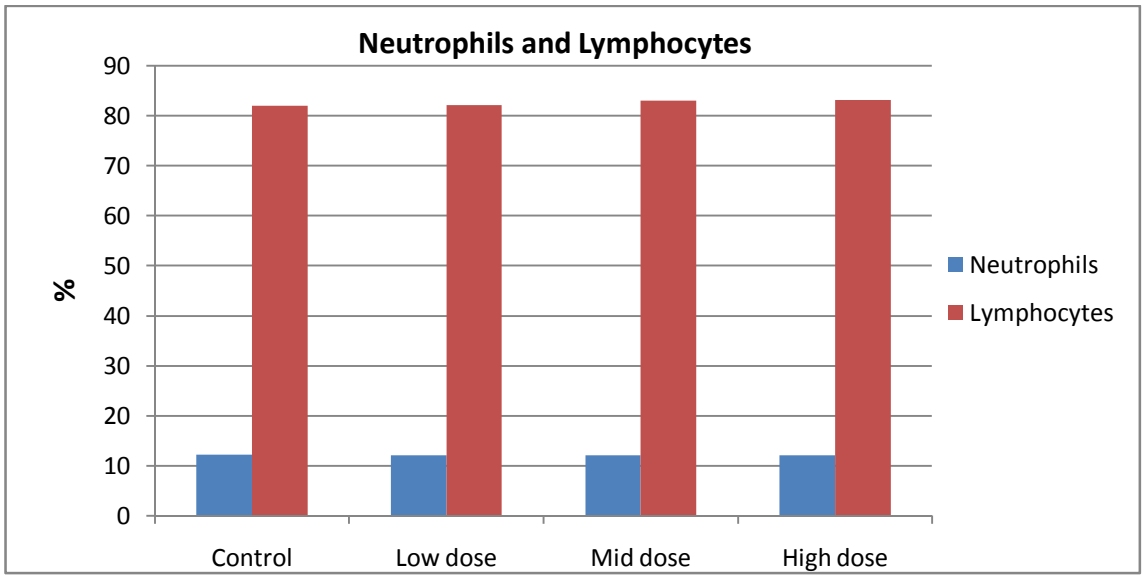
The mean value of T.WBC and Platelets of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart- 2



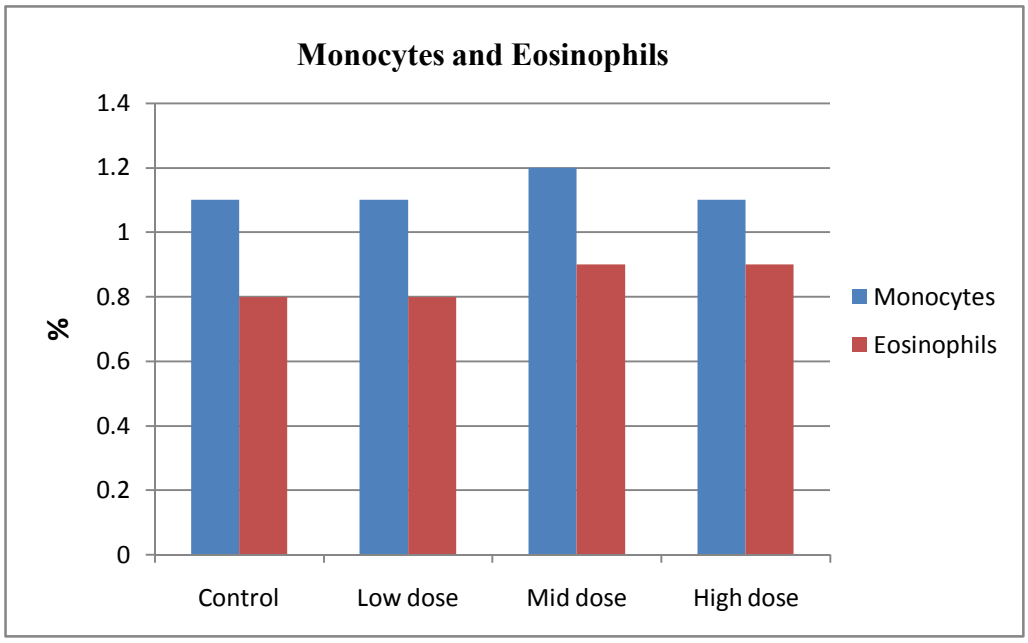
The mean value of Neutrophils and Lymphocytes of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart- 3



The mean value of Monocytes and Eosinophils of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart - 4



The mean value of PCV,MCV and MCHC of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart – 5

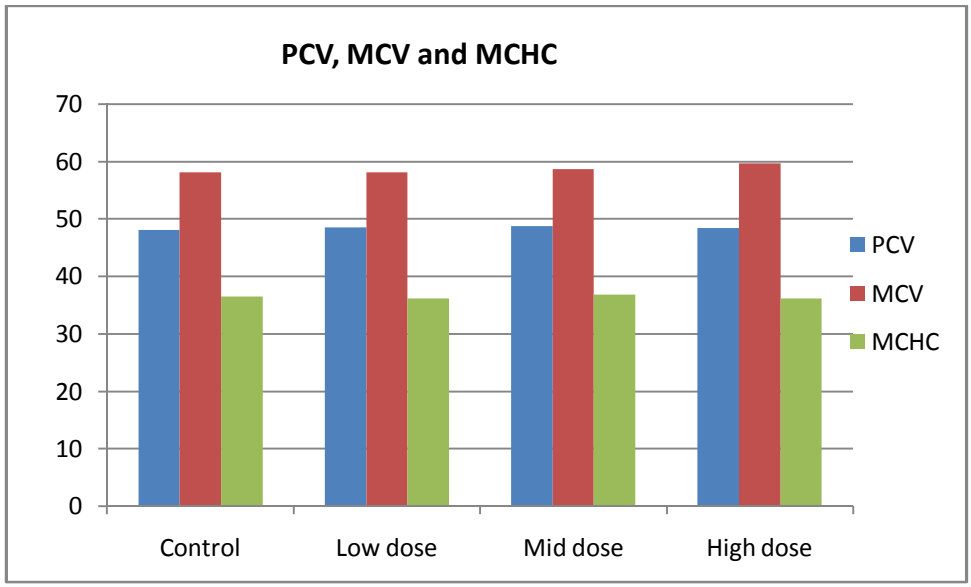


Table17: Biochemical Parameters of of Wistar albino rats group exposed to *Panchakkini Chenduram*

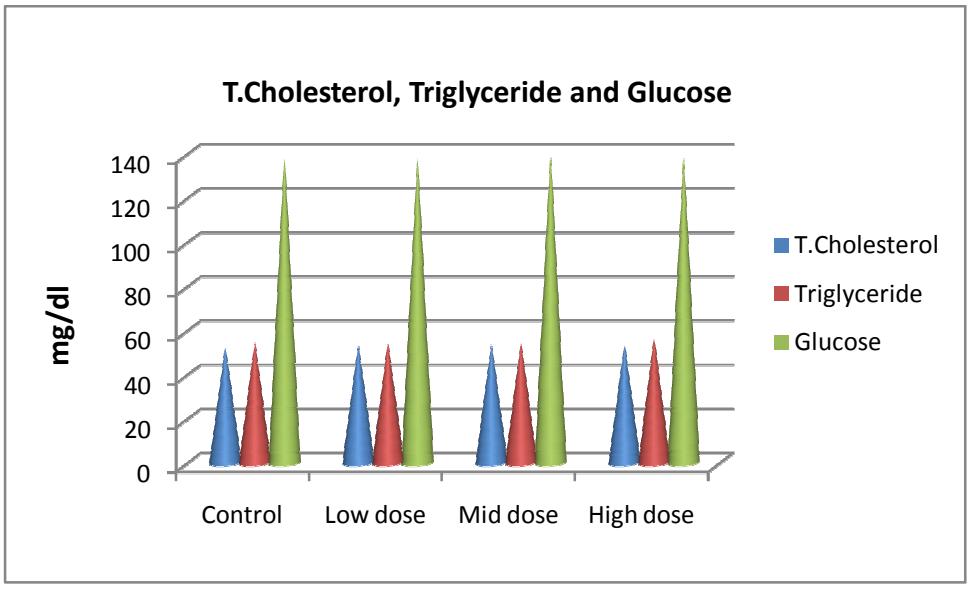
BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
GLUCOSE (R) (mg/dl)	138.10±2.02	138.12±2.10	138.9±12.6	138.12±5.25	N.S
T.CHOLESTEROL(mg/dl)	52.14±5.10	53.15±5.20	53.40±1.68	53.21±1.10	N.S
TRIGLY(mg/dl)	54.15±1.82	54.11±1.32	54.15±1.22	56.16±1.21	N.S
LDL	39.6±2.13	39.7±2.05	39.10±1.03	39.40±01.32	NS
VLDL	14.2±1.52	14.20±2.41	14.02±1.32	14.04±12.1	NS

				5	
HDL	12.12±4.32	12.32±2.50	12.46±1.20	12.51±1.23	NS
Ratio 1(T.CHO/HDL)	3.73±1.16	3.72±1.80	3.73±1.32	3.74±2.33	NS
Ratio 2(LDL/HDL)	1.92±1.22	1.92±1.20	1.93±2.20	1.94±006.02	NS
Albumin (g/dL)	4.21±0.22	4.22±0.52	4.4±7.20	4.55±6.48	NS

NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

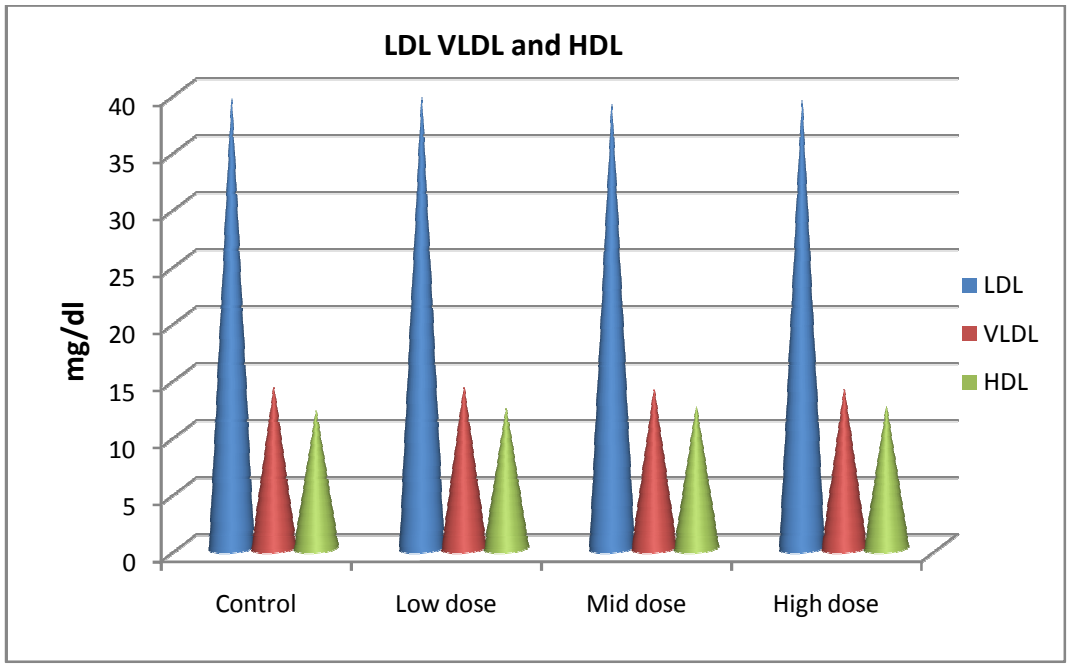
The mean value of T.Cholesterol, Triglyceride and Glucose of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart– 6



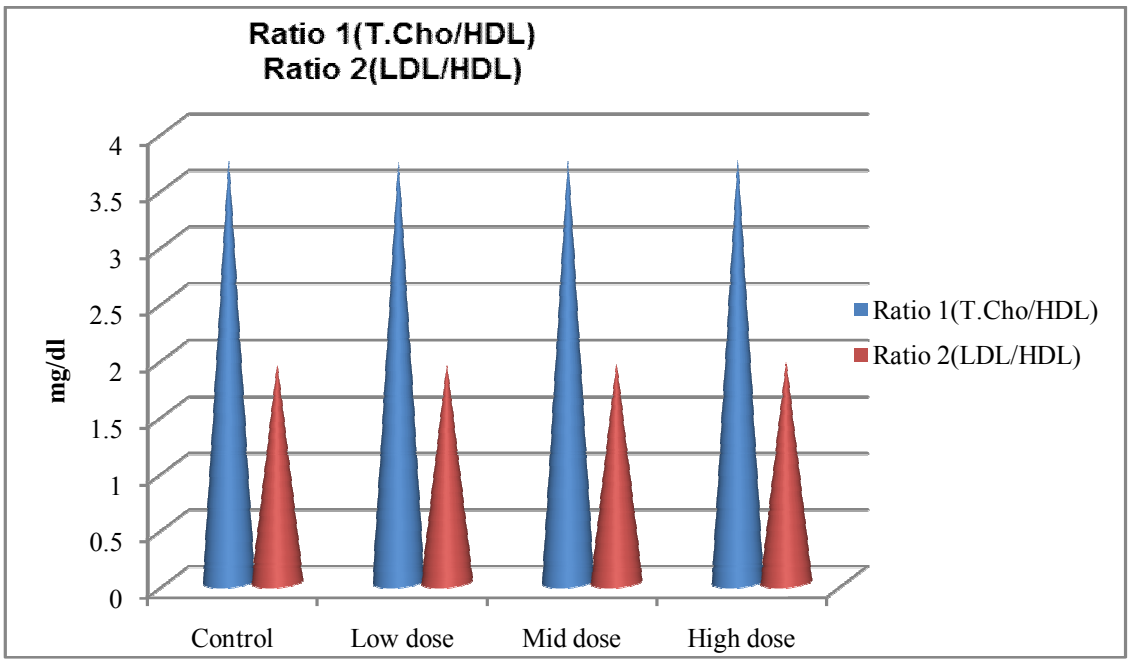
The mean value of LDL VLDL and HDL of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart – 7



The mean value of Ratio 1 (T.Cho/HDL) and radio to LDL / HDL of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart – 8



The mean value of Albumin of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart - 9

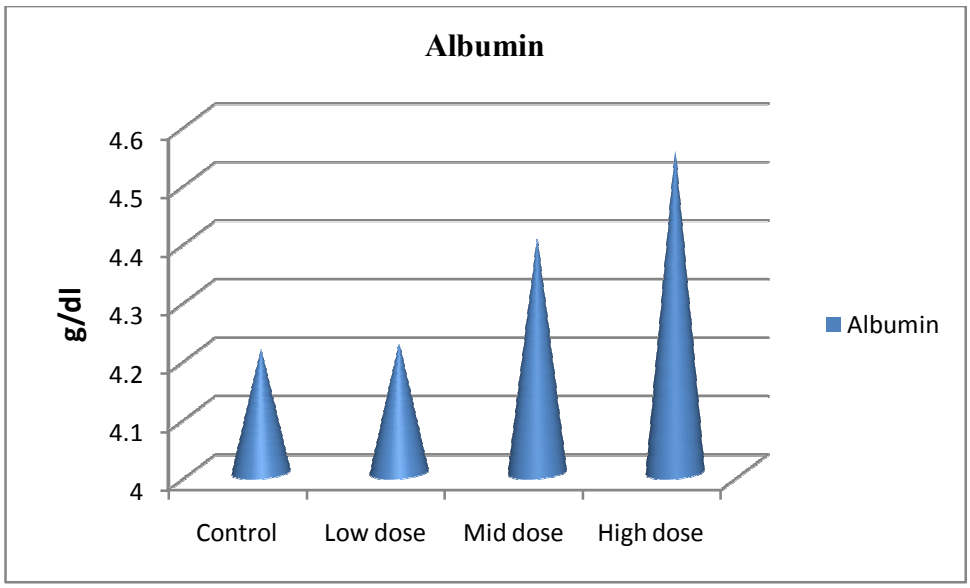


Table 18: Renal function test of of Wistar albino rats group exposed to*Panchakkini Chenduram*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
UREA (mg/dl)	15.50±0.29	15.50±0.29	15.46±1.18	15.42±1.22	N.S
CREATININE(mg/dl)	0.58±0.02	0.60±0.04	0.58±0.03	0.58±0.09	N.S
BUN(mg/dL)	19.1±0.02	19.10±0.34	19.6±0.42	19.26±1.02	NS
URIC ACID(mg/dl)	1.02±0.04	1.06±0.21	1.4±0.12	1.20±0.10	N.S

NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

The mean value of Urea, Creatinine, BUN and Uric acid of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart - 10

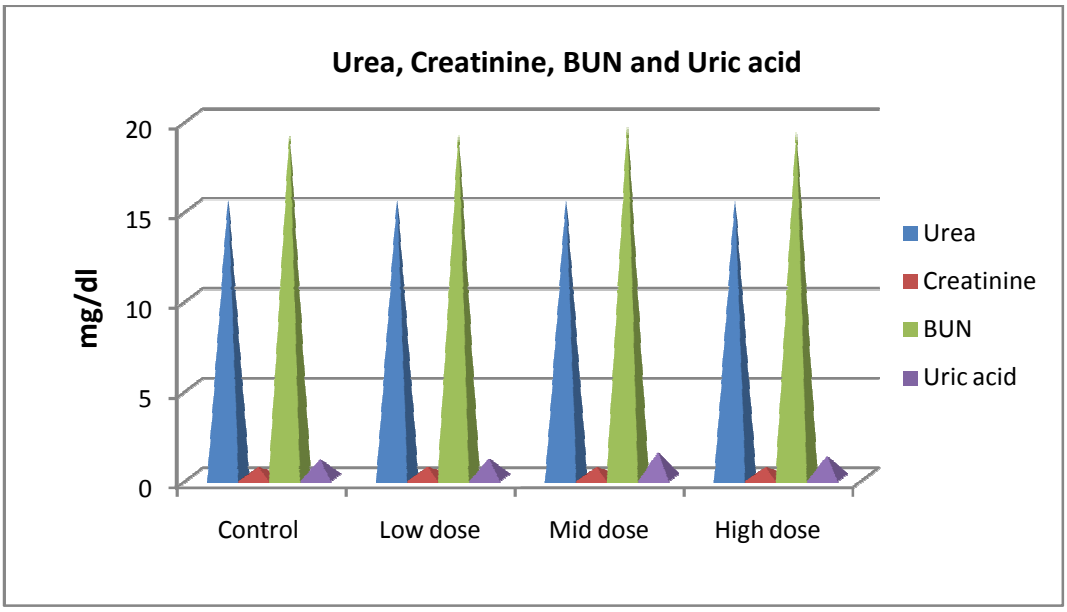


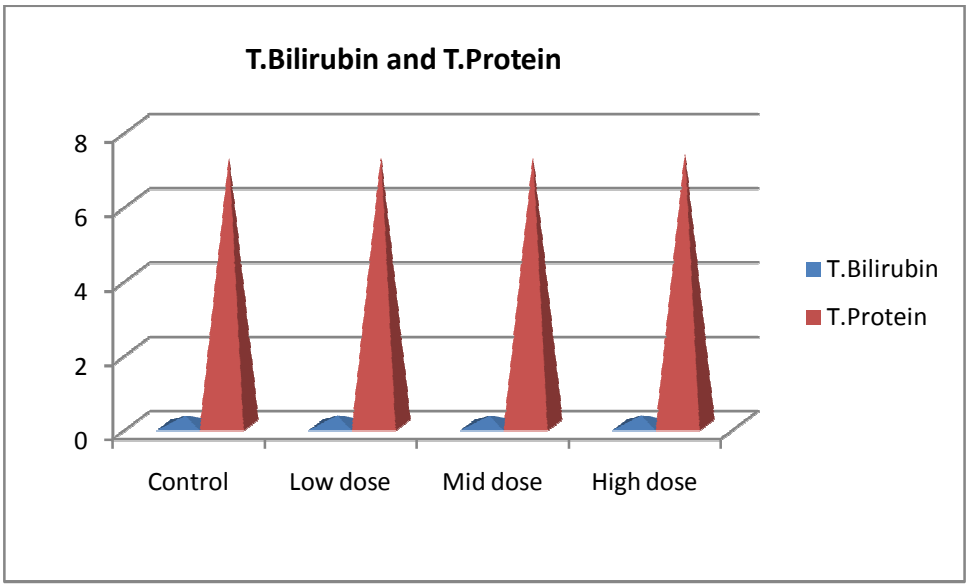
Table 19: Liver Function Test of Wistar albino rats group exposed to *Panchakkini Chenduram*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
T. BILIRUBIN (mg/dl).	0.25±0.01	0.26±0.03	0.25±0.03	0.26±0.01	N.S
SGOT/AST(U/L)	114.11±1.53	114.12±0.22	114.24±1.54	114.74±1.53	N.S
SGPT/ALT(U/L)	34.21±1.02	33.34±1.04	34.44±1.16	33.38±0.21	N.S
ALP(U/L)	112.11±2.21	112.22±2.20	112.23±1.24	112.03±6.02	N.S
T.PROTEIN(g/dL)	7.2.40±0.14	7.2±0.41	7.2±0.60	7.3±0.61	N.S

NS- Not Significant, **($p > 0.01$), * ($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

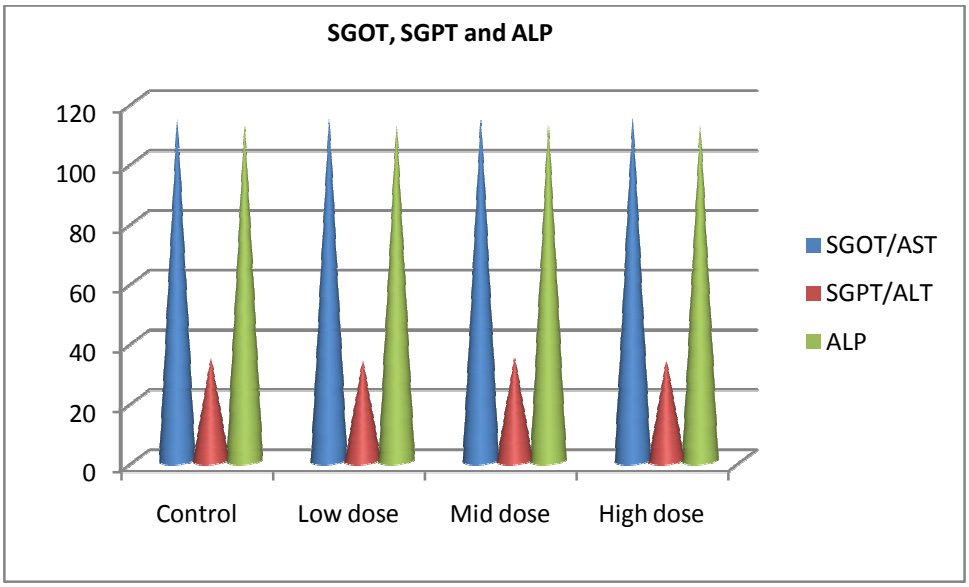
The mean value of T.Bilirubin and T.Protein of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart - 11



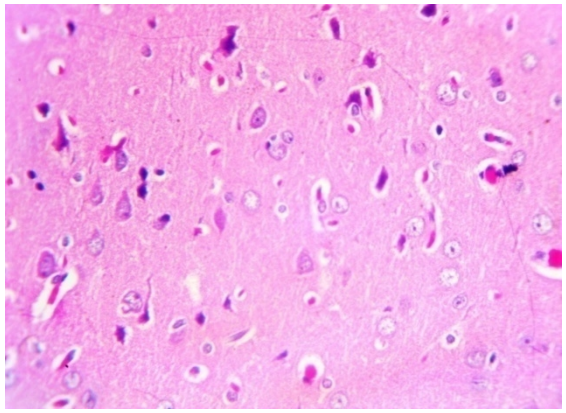
The mean value of SGOT/AST, SGPT/ALT and ALP of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 28 Days toxic study.

Chart - 12

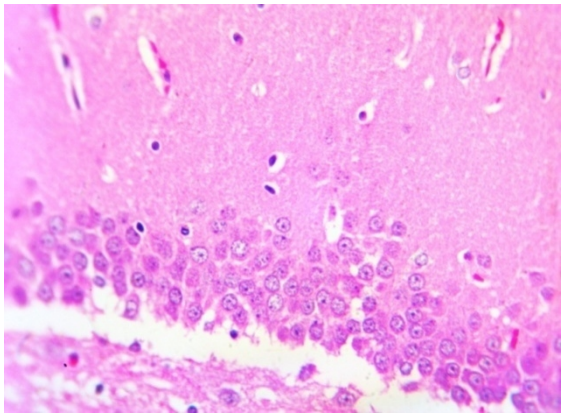


HISTOPATHOLOGY OF VITAL ORGANS

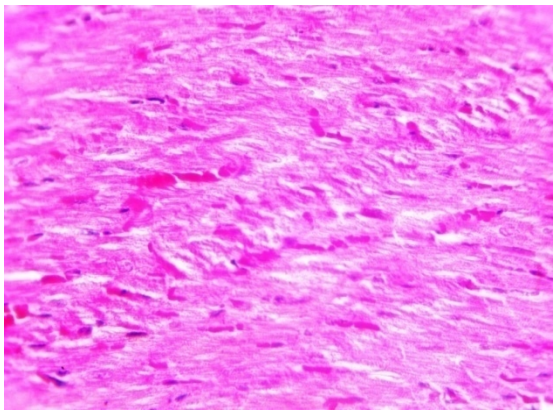
Control **High power Magnification 40x** **High Dose**



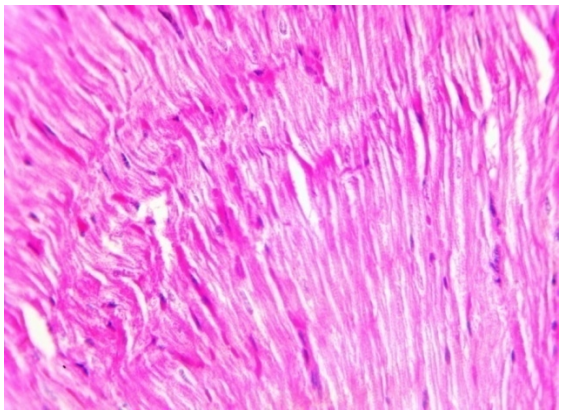
Brain



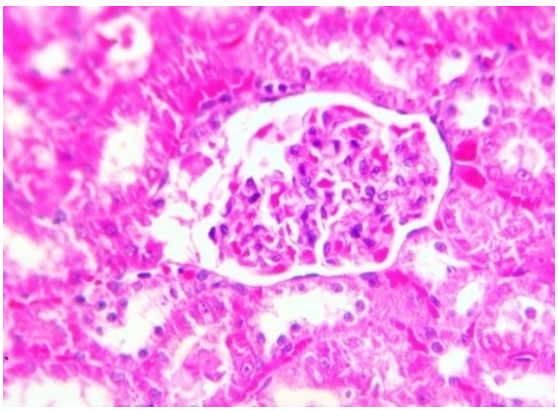
Brain



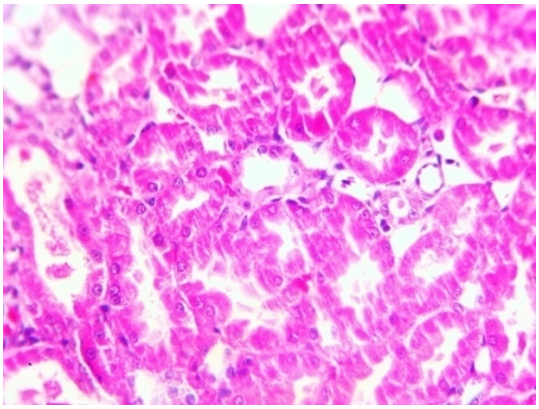
Heart



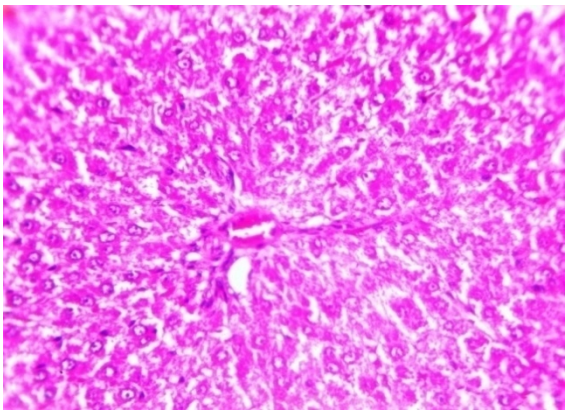
Heart



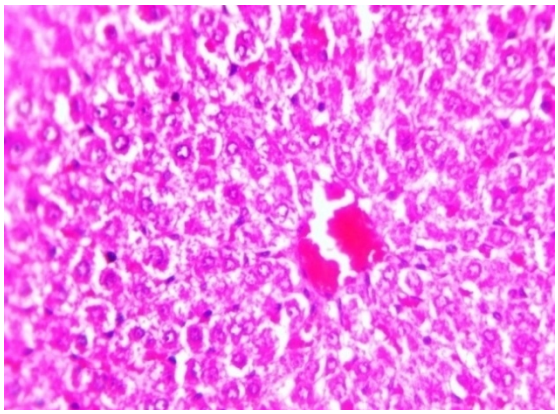
Kidney



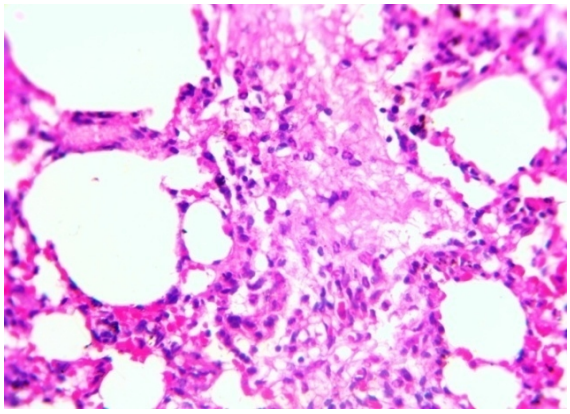
Kidney



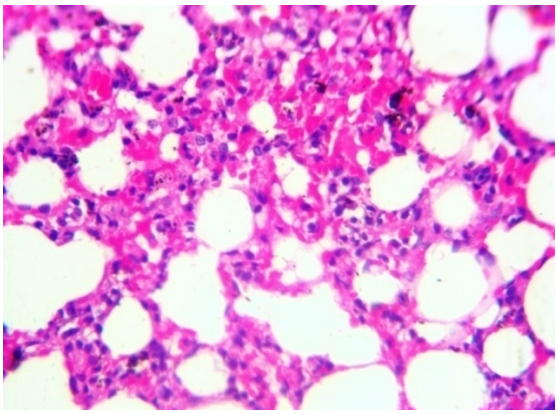
Liver



Liver



Lungs



Lungs

Interpretation:

Liver:

Section of liver from control animals showed No degeneration of hepatocytes, focal steatosis, and congestion of central vein and inflammation of portal tract. Liver of treated groups showed No degeneration of hepatocytes, focal steatosis, and congestion of central vein and inflammation of portal tract

Kidney:

Section of kidney from control animals showed normal size of glomeruli with normal tubules. Kidney of treated animals showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney.

Lungs:

Section of lungs from control animal showed normal architecture of a section and the drug treated group shows mild paraseptal thickening with inflammation

Brain:

Section of brain from control animals showed brain cortex with normal architect. Treated animals showed brain cortex with normal architecture.

Heart:

Section of heart from control animal showed normal muscle fibers with acidophilic cytoplasm and centrally located nuclei

In treated group showed normal muscle fibers with acidophilic cytoplasm and centrally located nucleiwith normal structure.

Heart, Lungs, brain, liver and kidney showed no cellular architecture in treated groups. From the histopathological study no related changes in vital organs are observed in treated groups.

Results:

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days for subacute toxicity study. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 days. During dosing period, the quantity of food and water consumed by animals from different dose groups was found to be comparable and normal with that by control animals.

The results of haematological investigations conducted on day 29th day revealed no significant changes in the haematological values when compared with those of respective controls. This gave clear justification that bone marrow and spleen were not influenced by *Panchakkini Chenduram*.

Results of Biochemical investigations conducted on days 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT,SGPT, Bilirubin were within the limits, blood glucose significant compared to control group.

The other cardio vascular risk markers were also within normal ensured that *Panchakkini Chenduram* did not influence the Cardio vascular system.

Group Mean Relative Organ Weights are recorded Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable with respective control group. Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities.

The vital organs such as liver, heart, kidneys, lungs and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control. According to these results, *Panchakkini Chenduram* could be concluded as no-observed-adverse-effect level (NOAEL). It showed the safety of the drug which proved its utility in long time administration without any harm to the human being.

RESUIT OF REPEATED DOSE 90 DAYS ORAL TOXITY STUDY IN WISTAR RATS

Table 20: Body weight of Wistar albino rats group exposed to *Panchakkini Chenduram*

DOSE	DAYS						
	1	15	30	45	60	75	90
CONTROL	300.2±12.03	301.2 ± 14.14	301.6 ± 20.42	302.4 ± 13.50	302.5 ± 12.20	304±16.20	304±15.02
LOW DOSE	310.2 ± 20.20	310.5 ± 2.24	312.4± 12.21	322 ± 04.12	323.02± 20.50	324±03.20	326±12.32
MID DOSE	308.4± 32.50	308.3 ± 20.05	308.4 ± 20.20	309.4 ± 10.20	310.6 ± 20.02	310±22.14	310±16.26
HIGH DOSE	280± 12.10	280 ± 12.30	280±04.22	281 ± 30.16	281.32 ± 04.10	281±11.10	282±16.34
P value (p)*	NS	NS	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Table 21: Water intake (ml/day) of Wistar albino rats group exposed to *Panchakkini Chenduram*

DOSE	DAYS						
	1	15	30	45	60	75	90
CONTROL	98.4 ± 1.25	98±1.02	98.8±2.30	98±5.12	98.5±1.06	99.25±7.16	99±77.50
LOW DOSE	97.2±6.40	97.4±8.50	97.6±1.14	98.2±1.40	98.4±1.50	98.4±1.56	98±2.84
MID DOSE	95.4±2.10	95.3±2.24	95.12±2.02	96.4±2.17	96.5±1.32	96.4±1.50	96.2±1.42
HIGH DOSE	87.1±1.30	87.2±2.20	87.7±4.52	87.2±1.82	87.4±6.78	88±4.51	89±2.20
P value (p)*	NS	NS	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Table 22: Food intake (gm/day) of Wistar albino rats group exposed to *Panchakkini Chenduram*

DOSE	DAYS						
	1	15	30	45	60	75	90
CONTROL	224.6±1.22	225±5.04	225.6±6.20	226±4.96	228.2±2.12	229.32±1.26	229±24.32
LOWDOSE	221.2±1.22	221.2±1.42	221.4±3.13	221.2±2.14	221.8±1.22	222.8±2.23	223.6±2.54
MID DOSE	242.7±4.33	243.3±2.21	244.14±2.02	244.4±1.42	245.2±2.20	246.1±2.20	247±2.12
HIGHDOSE	246.1±1.43	246.2±2.61	246.6±2.40	247.2±1.20	247.4±2.40	248±2.24	248±2.80
P value (p)*	NS	NS	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Table 23: Haematological parameters of Wistar albino rats group exposed to *Panchakkini Chenduram*

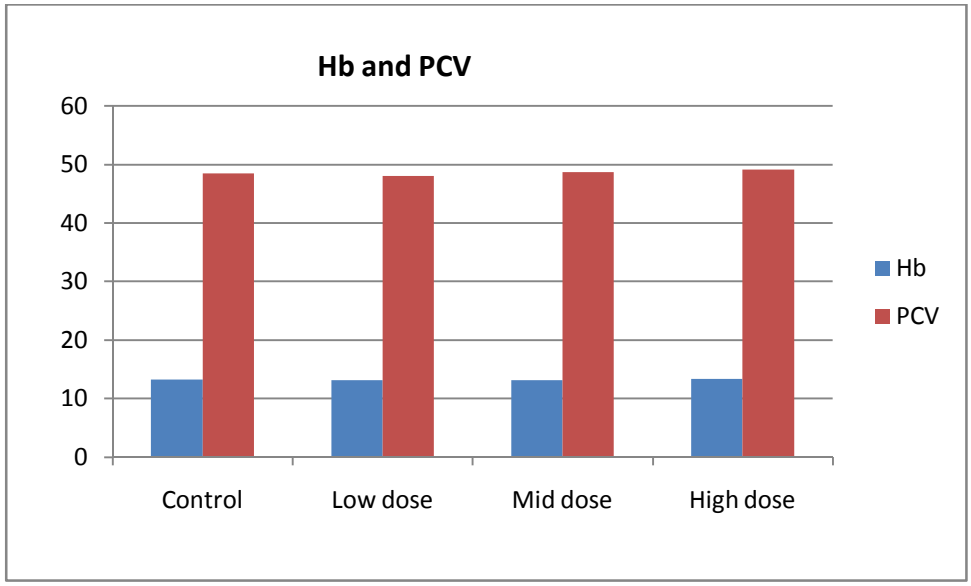
Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin(g/dl)	13.2±0.20	13.10±0.33	13.15±0.76	13.38±0.46	N.S
Total WBC ($\times 10^3$ l)	13.92±0.05	13.62±0.33	13.94±0.20	13.40±9.06	N.S
Platelets cells10^3/µl	724.15±2.26	724.17±4.76	725.19±3.62	725.05±2.14	N.S
Neutrophils(%)	11.12±0.04	11.15±0.60	11.25±1.54	11.10±2.40	N.S
Lymphocyte (%)	85.12±1.21	85.20±1.14	85.21±1.06	85.15±1.26	N.S
Monocyte (%)	2.45±0.02	2.87±0.04	2.56±0.06	2.87±0.07	N.S
Eosinophil(%)	0.80±0.04	0.81±0.07	0.83±0.02	0.80±0.03	N.S
Total RBC 10^6/µl	7.42±0.03	7.64±0.70	7.24±0.27	7.82±0.32	N.S

PCV%	48.50±0.6	48.10±1.20	48.70±1.52	49.16±2.14	N.S
MCHC g/Dl	36.4±1.42	36.6±0.72	36.7±2.80	36.30±1.74	N.S
MCV fL(µm^3)	53.04±4.60	53.06±3.30	53.20±2.32	54.24±2.24	N.S

N.S- Not Significant, **($p > 0.01$), *($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

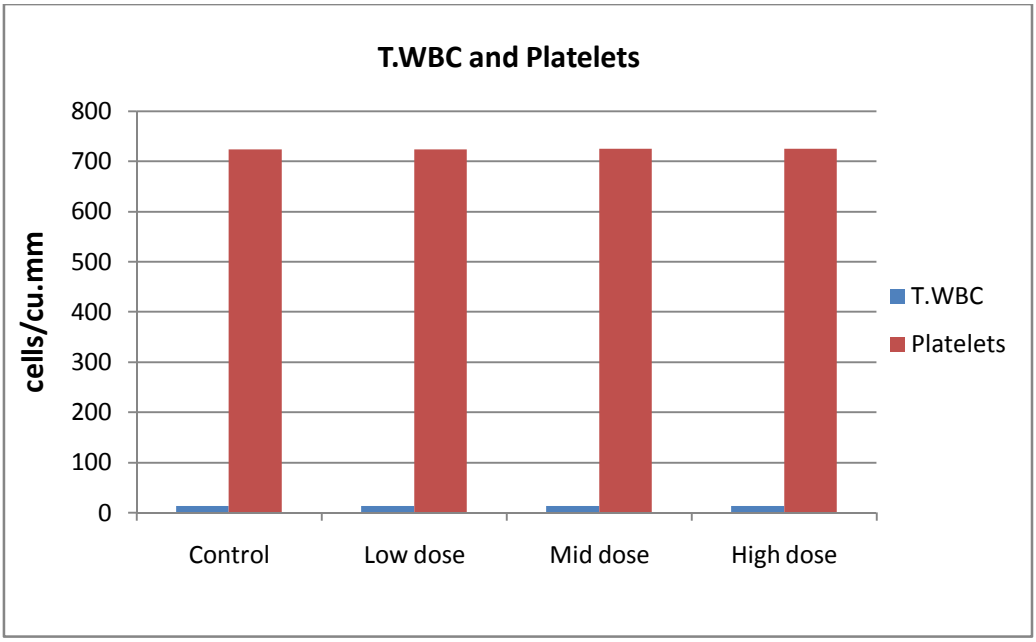
The mean value of Hb and PCVof control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

Chart- 13



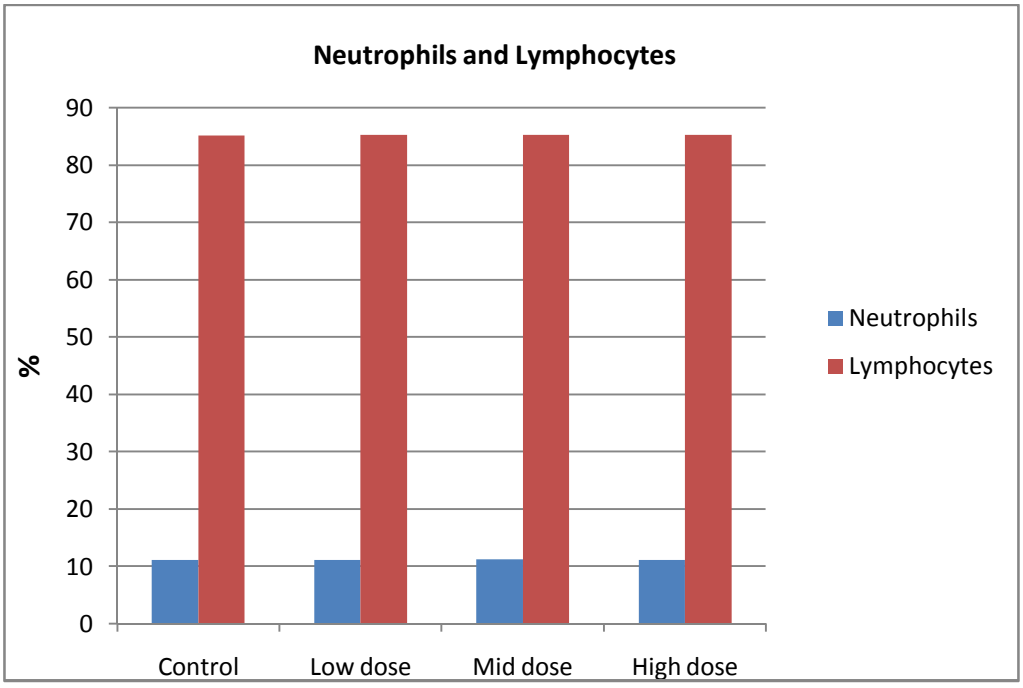
The mean value of T.WBC and Platelets of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

Chart- 14



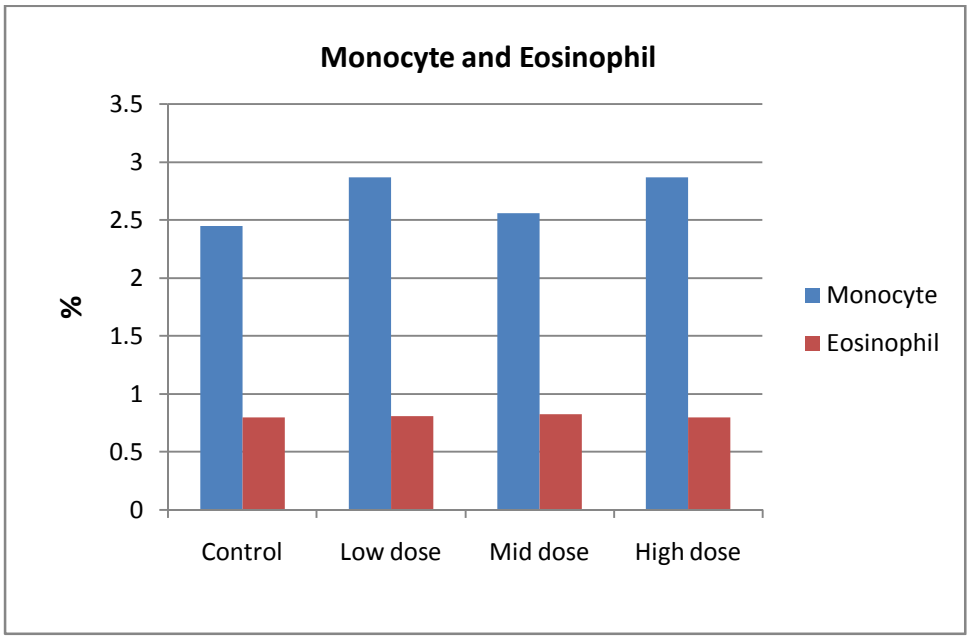
The mean value of Neutrophils and Lymphocytes of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

Chart- 15



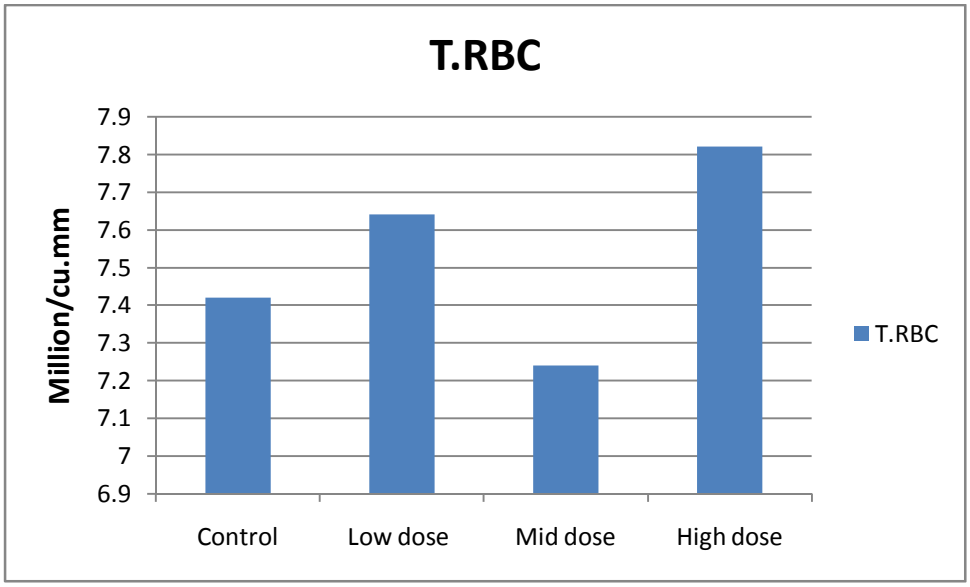
The mean value of Monocyte and Eosinophil of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

Chart- 16



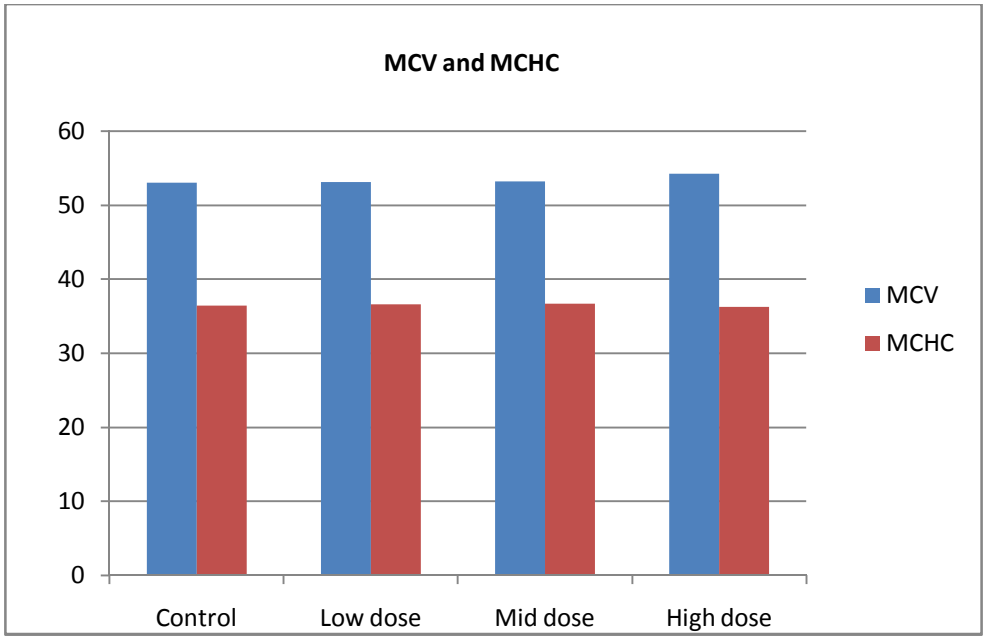
The mean value of T.RBC of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 days toxic study.

Chart- 17



The mean value of MCV and MCHC of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

Chart- 18



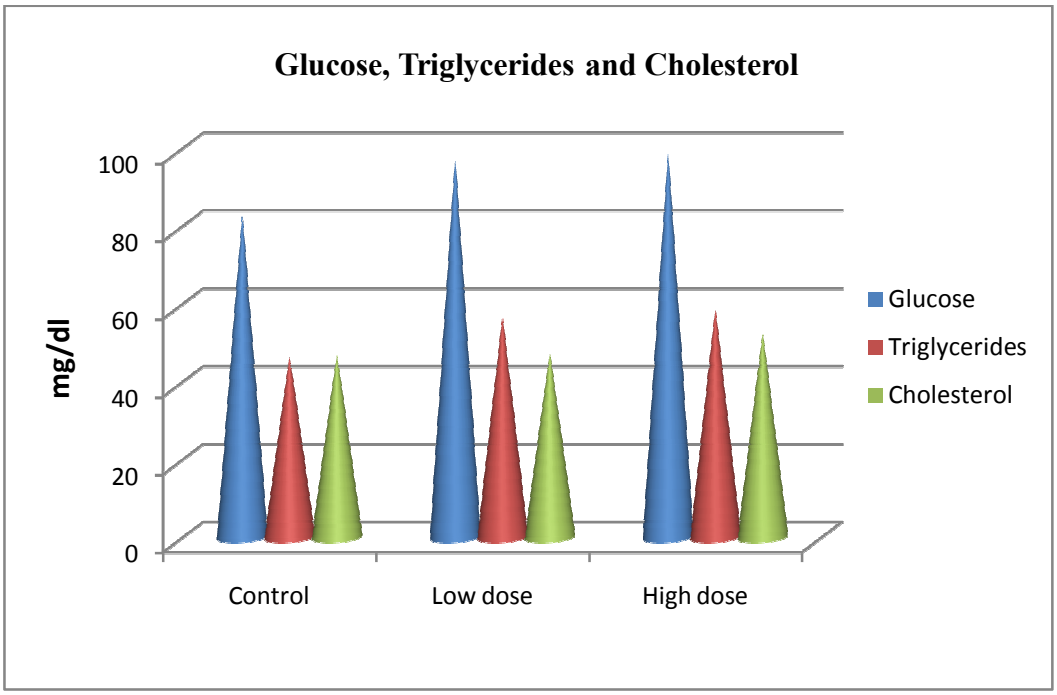
Tab24: Liver Function Test of Wistar albino rats group exposed to *Panchakkini Chenduram*

Group	Treatment	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	SGPT (U/L)	ALP (U/L)
I	Control	82.64±2.11	46.24±09.32	46.81±4.45	52.22±3.39	215.08±12.48
II	Low dose	96.84±10.24	56.34±6.22	47.04±2.43	70.87±6.57	280.60±17.68
III	High dose	98.63±09.24	58.44±7.74	52.20±6.46	82.11±1.24	312.44±12.94

NS- Not Significant, **($p > 0.01$), * ($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

The mean value of Glucose, Triglycerides and Cholesterol of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

Chart- 19



The mean value of SGPT and ALP of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

Chart- 20

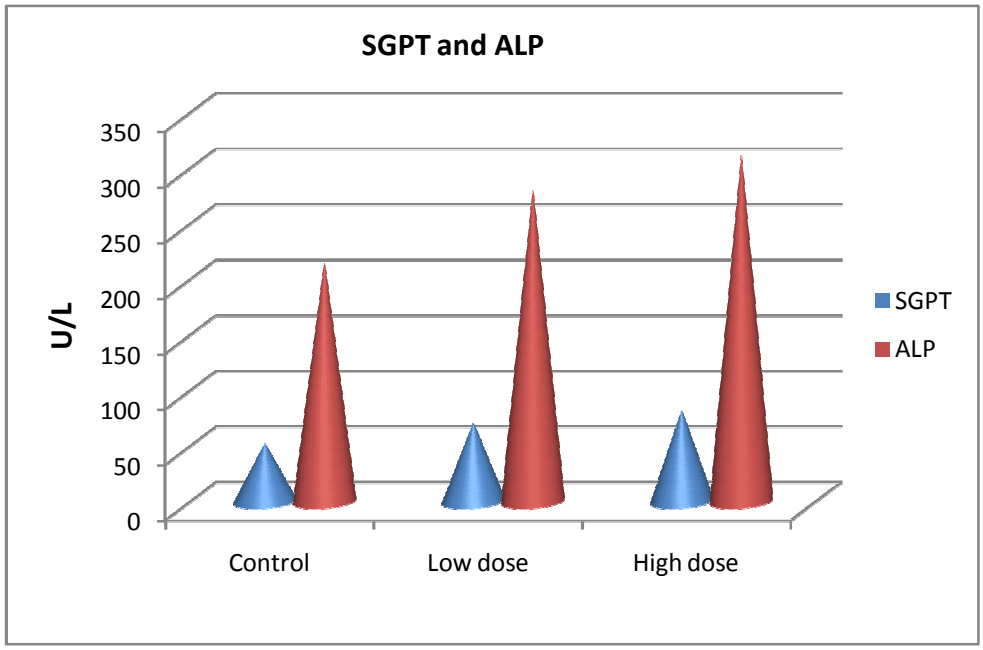
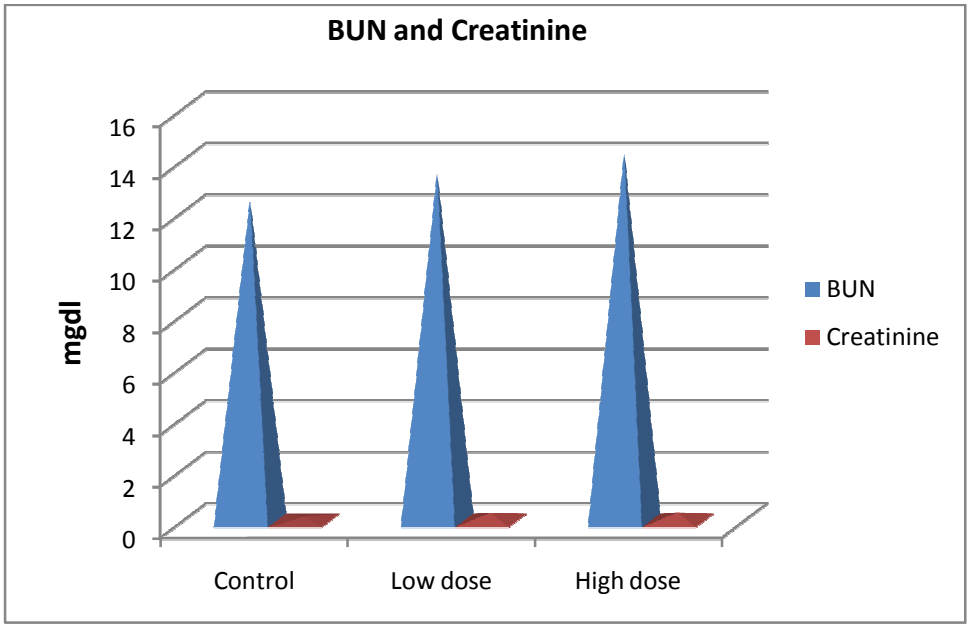


Table 25: Biochemical Parameters of of Wistar albino rats group exposed to *Panchakkini Chenduram*

Group	Treatment	BUN (mg/dL)	LDL (U/L)	T. Protein (g/dL)	Albumin (g/dL)	Creatinine (mg/dL)
I	Control	12.40±0.20	318.18±44.15	2.36±0.42	0.70±0.04	0.14±0.04
II	Low dose	13.46±0.90	348.40±18.19	3.84±0.24	1.33±0.19	0.31±0.04
III	High dose	14.24±1.22	390.10±42.03	4.14±0.12	1.85±0.11	0.32±0.02

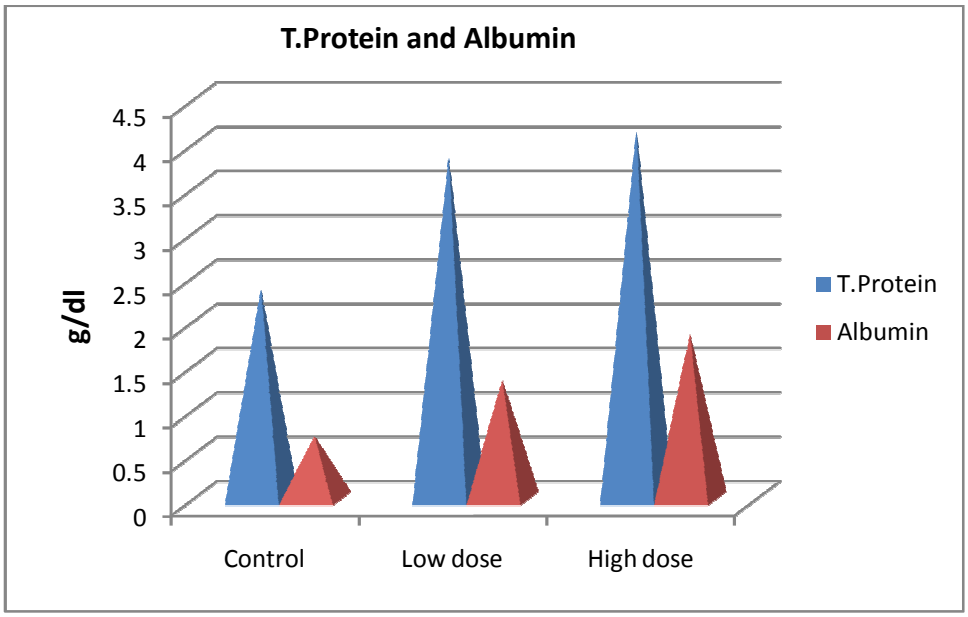
The mean value of BUN and Creatinine of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

Chart- 21



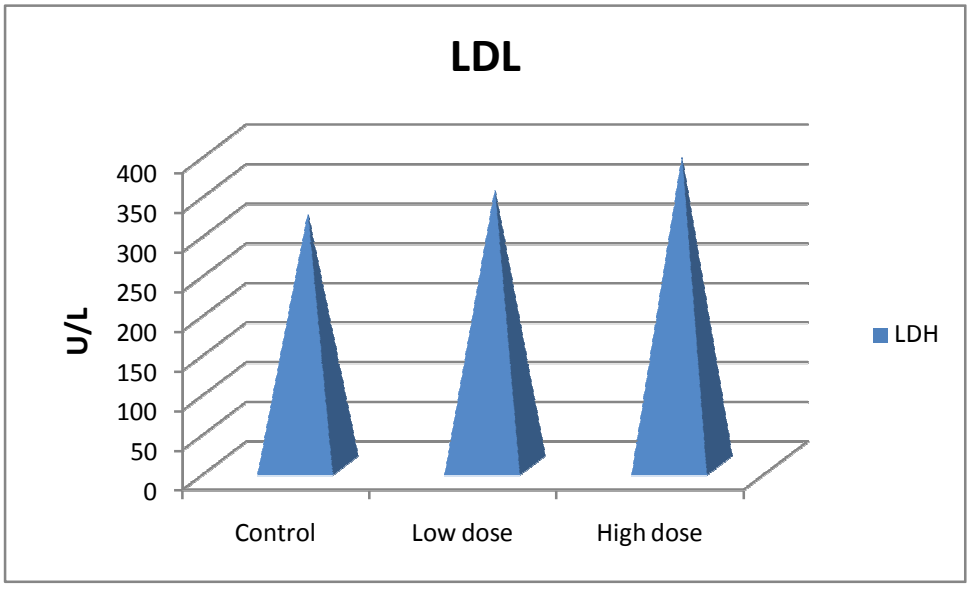
The mean value of T.Protein and Albumin of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

Chart- 22



The mean value of LDL of control and treated groups of Wistar albino rats exposed to *Panchakkini Chenduram* in repeated oral 90 Days toxic study.

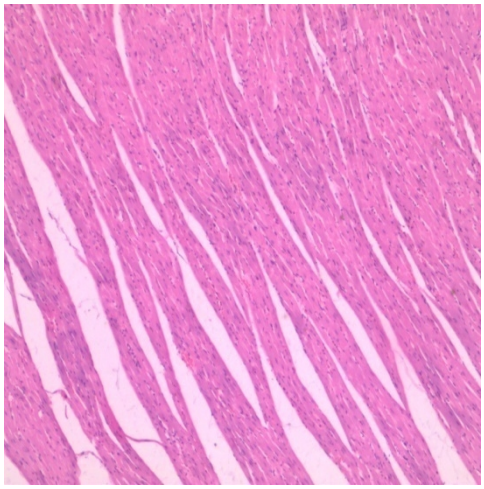
Chart- 23



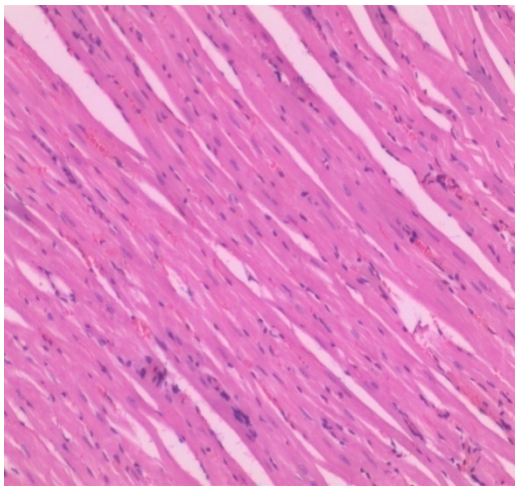
Histopathology of Control group animals

Heart

Low Power Magnification 10X

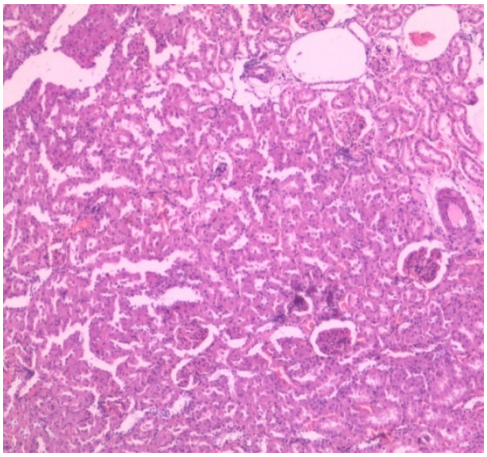


High Power Magnification40X

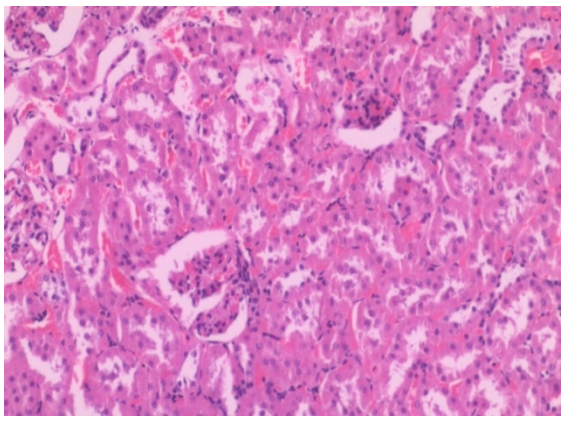


Kidney

Low Power Magnification 10X

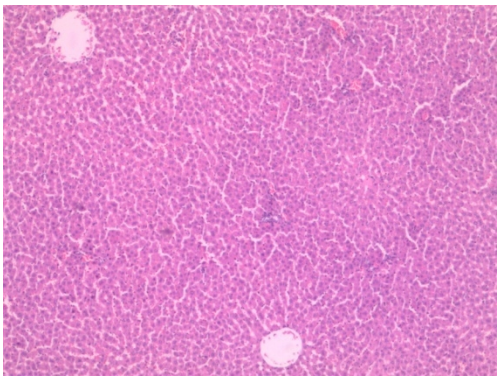


High Power Magnification 40X

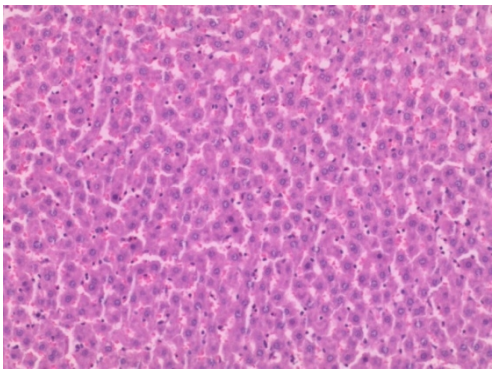


Liver

Low Power Magnification 10X

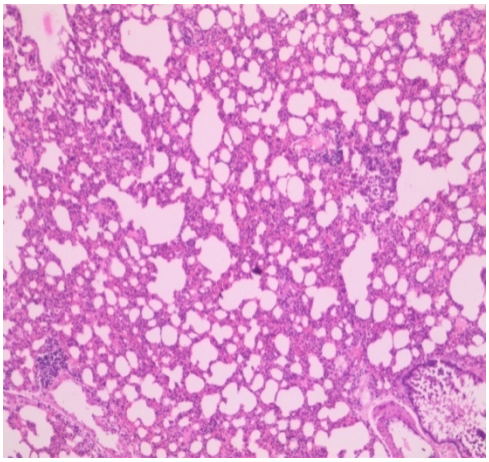


High Power Magnification 10X



Lung

Low Power Magnification 10X



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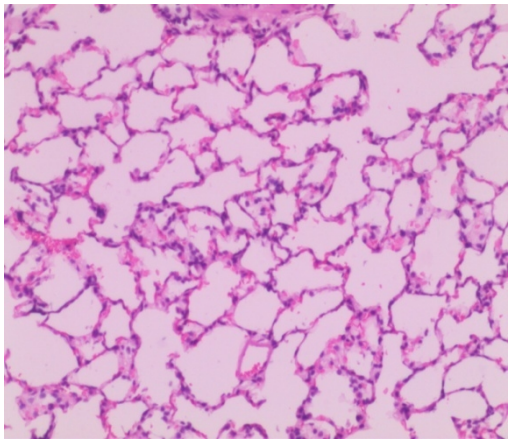
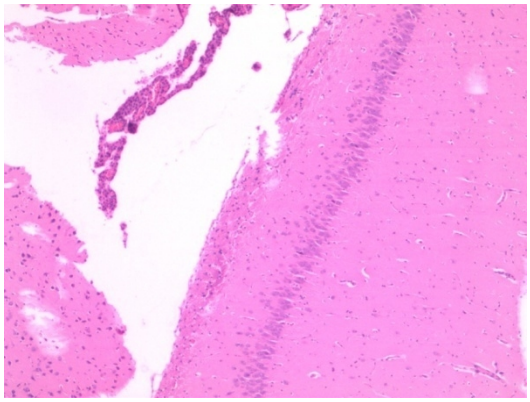
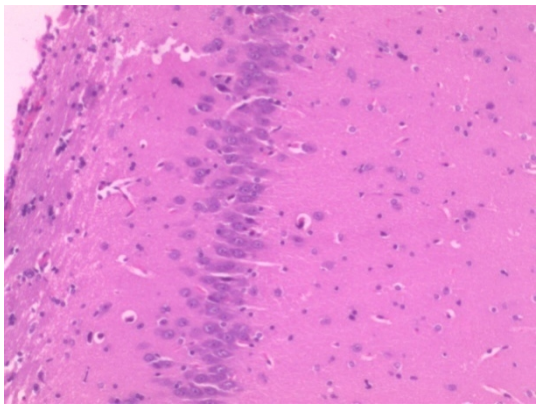


Brain

Low Power Magnification 10X



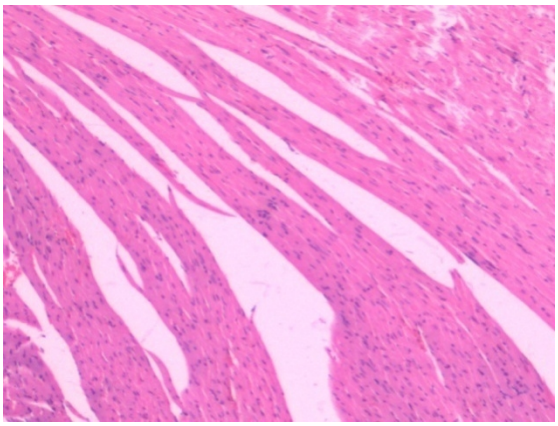
High Power Magnification 40X



Histopathology of High dosed group animals

Heart

Low Power Magnification 10X



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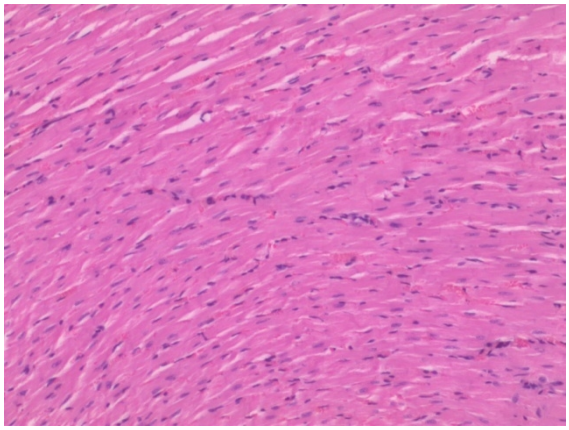
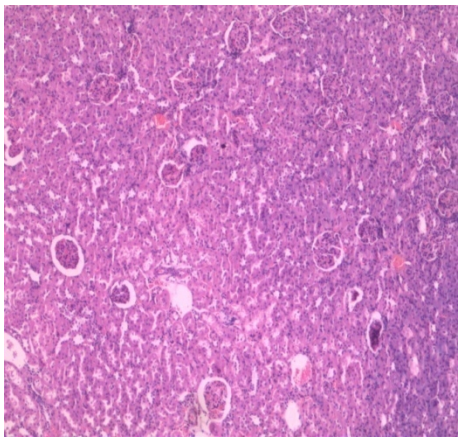


Kidney

Low Power Magnification 10X



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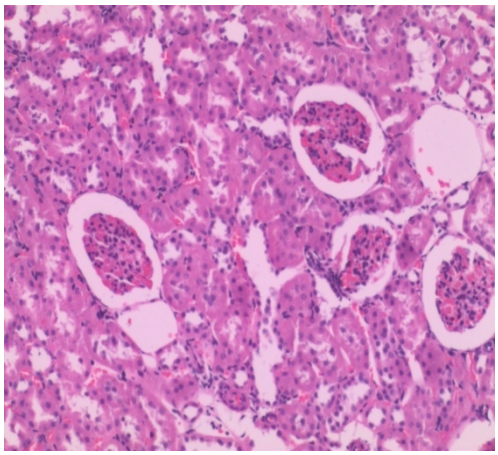
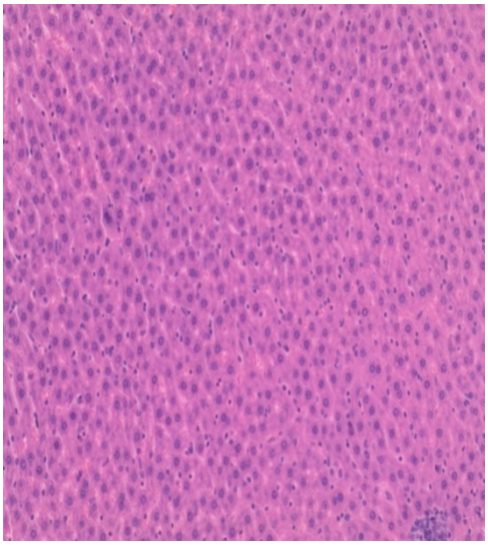


Liver

Low Power Magnification 10X



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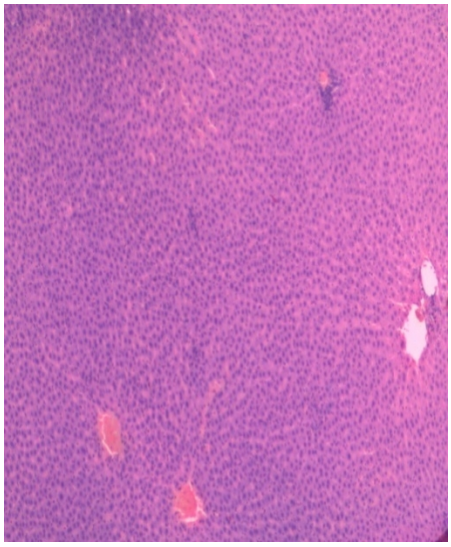
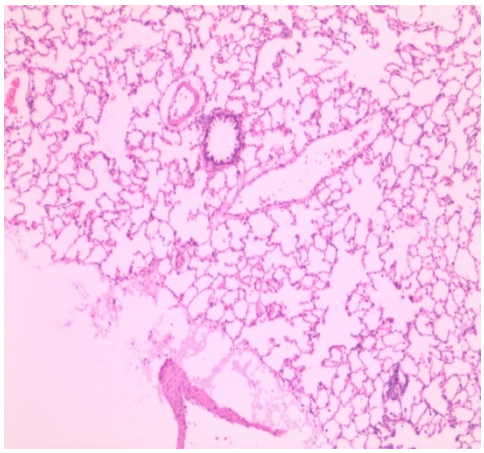


Lung

Low Power Magnification 10X



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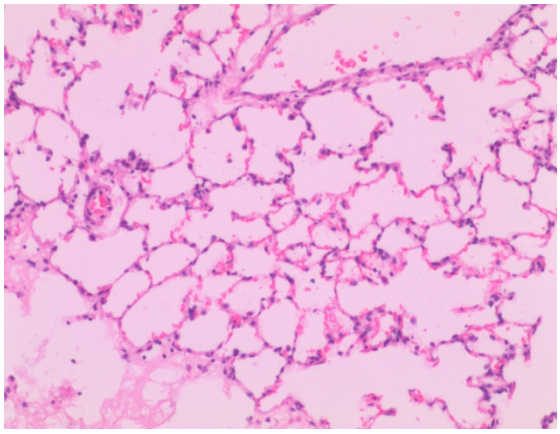
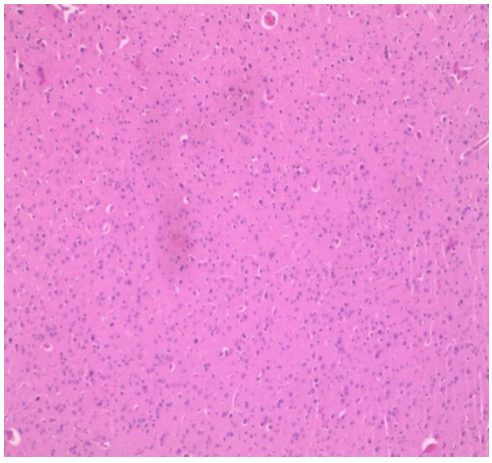
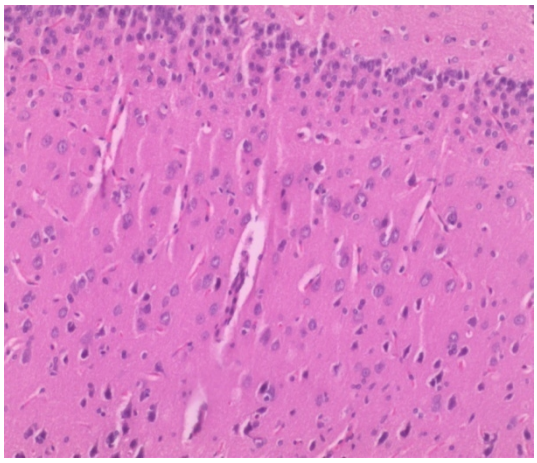


Brain

Low Power Magnification 10X



High Power Magnification 40X



Interpretation

Kidney

- Appearance of glomeruli, tubules, interstitium and lumen was normal in both the samples with no signs of degeneration
- Interstitial connective tissue of both the sample appear normal

Heart

- Perfectly -arranged myocardial fibers, clear transverse striation and normal structure were observed.
- Appearance of cardiomyocyte was normal with dark nuclear region. The nuclei of muscle fibers appear oval arrangement.

Liver

- Hepatocyte appears with dark pigment chromatin in centri lobular and periportal region
- Hepatic sinusoid and hepatic cord was normal

Lung

- Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis
- Perivascular region appears normal, Alveolar septa and wall appeared widen and normal
- No signs of lymphocyte cuffing
- No signs of airway secretion and bronchial secretion
- Bronchial blood vessels and connective tissue appears normalwith no signs of pulmonary edema

Brain

- Arrangement of the neurons appears intact with no signs of degeneration or apoptotic changes in both the samples
- Cortex region showed normal neurons with polygonal to round cell bodies containing dense cytoplasm.
- No signs of ischemia or lesion were observed

Interpretation of sub-chronic toxicity study:

Sub-chronic oral toxicity repeated dose of *Panchakkini Chenduram* on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 days. Various parameters were studied and the interpretation of the study result is discussed below.

Body weight

The result of the body weight of rats exposed to control and the *Panchakkini Chenduram* of different dose groups exhibited overall weight gain throughout the dosing period of 90 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

Haematological investigation interpretation:

The haematological investigation results of the rats conducted on 91st day after the repeated dose of the drug revealed the values of different parameters.. The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Biochemical investigation interpretation:

The biochemical investigations were conducted on 90th day and the result is produced. The results revealed there are no significant changes in the values of different parameters with that of the control. But sugar levels were reduced significantly. other values were within the normal biological and laboratory limits.

Histopathology

Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities.

The vital organs such as liver, heart, kidneys, lungs and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control.

PHARMACOLOGICAL RESULTS OF HEPATO PROTECTIVE ACTIVITY

Table26: Effect of *Panchakkini chenduram* and Silymarin on haematological parameters of Paracetamol induced liver damage in rats

Parameters	Control	Control+ PCM 1g/kg	Silymarin 100 mg/kg+ PCM 1g/kg	PC 5mg/kg+ PCM 1g/kg	PC10mg/kg+ PCM 1g/kg
PCV (%)	44.31±1.76	21.37±1.26 ###	28.47±1.20	31.53±2.02 *	33.67±1.26 **
Hb (g/dl)	15.00±0.57	11.60±0.70###	12.67±0.33	13.53±0.17 **	13.43±0.23 ***
RBC (x 10^6/µ L)	6.33±0.17	5.30±0.11 ###	5.76±0.12*	6.333±0.18 *	6.033±0.26 **
Platelets (x 10^3/µ L	160.0±2.30	95.67±3.18 ###	114.17±2.40 **	124.3±1.76 **	125.7±2.90 ***
MCV	60.77±0.88	43.45±3.52 ###	45.70±2.08	52.33±1.20 *	54.30±2.00 *
MCH	23.13±1.85	13.63±1.20 #	12.87±0.88	14.33±1.76	15.27±2.40
MCHC	45.42±1.73	42.30±1.73 #	47.67±0.81	41.67±2.18	34.67±2.40
WBC X 10^3	6.73±0.59	8.87±0.17***	7.10±0.17	8.46±0.14 #	7.63±0.20 ###

PC – *PANCHAKKINI CHENDURAM* ***PCM- PARACETAMOL***

Values are Mean ± SEM; n = 6 animals in each group: # P<0.05, ## P< 0.01, ###P<0.001 is considered significant when compared with group I; *P<0.05, **P< 0.01, ***P<0.001 is considered significant when compared with group II by Tukey multiple comparison test.

Chart- 24

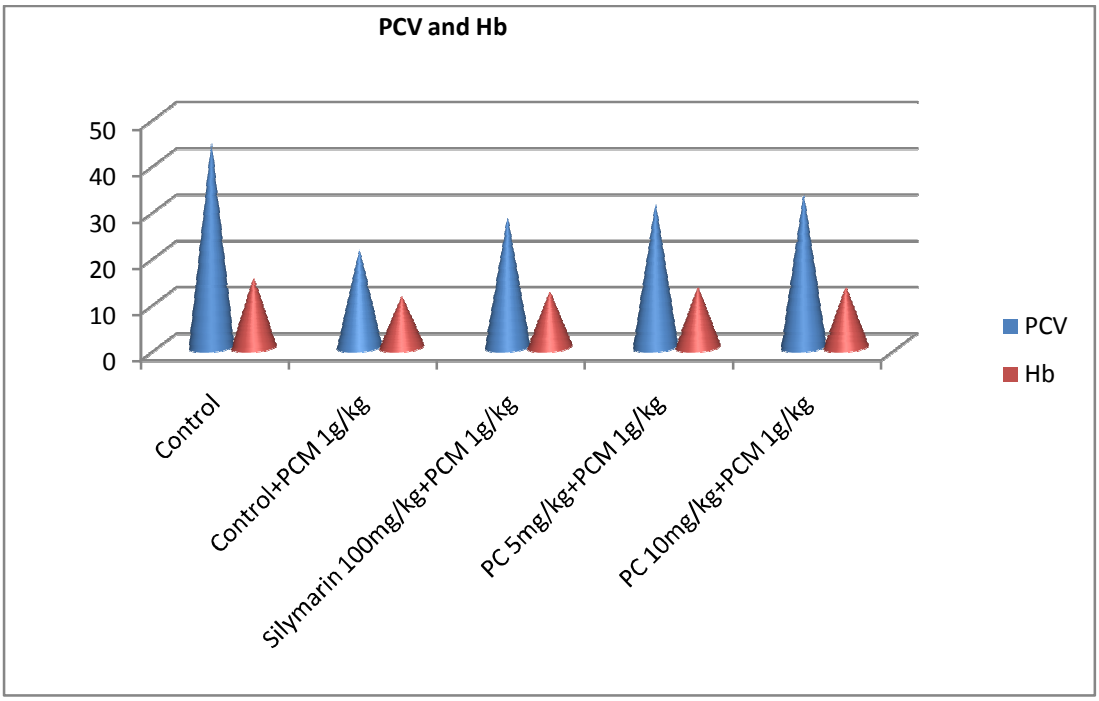


Chart- 25

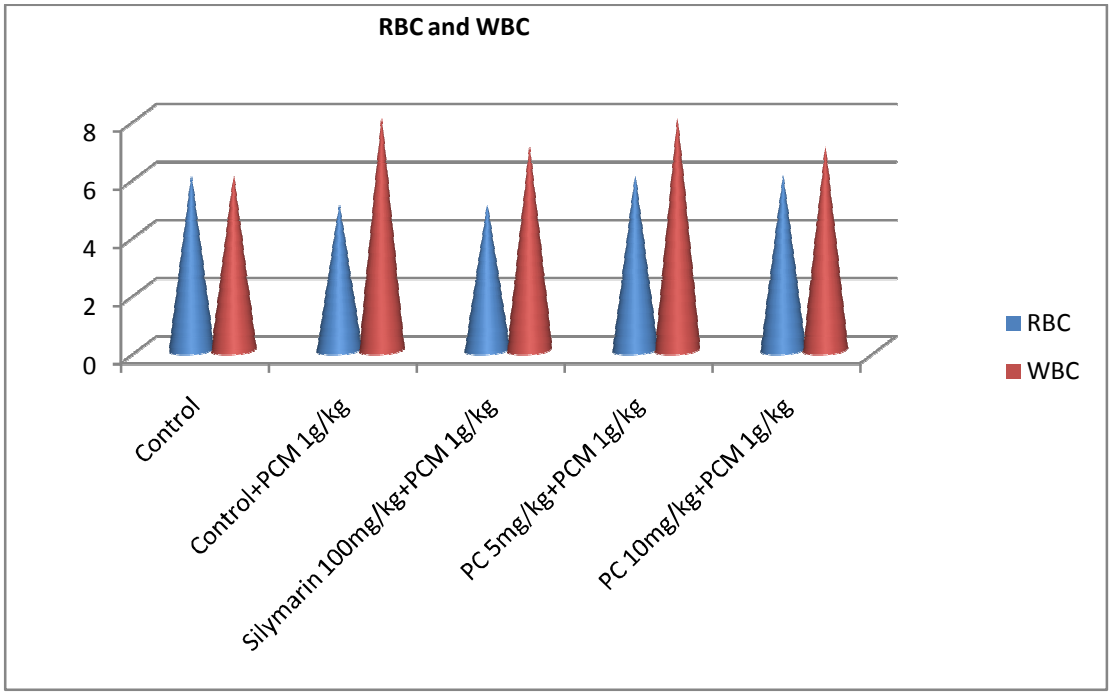


Chart-26

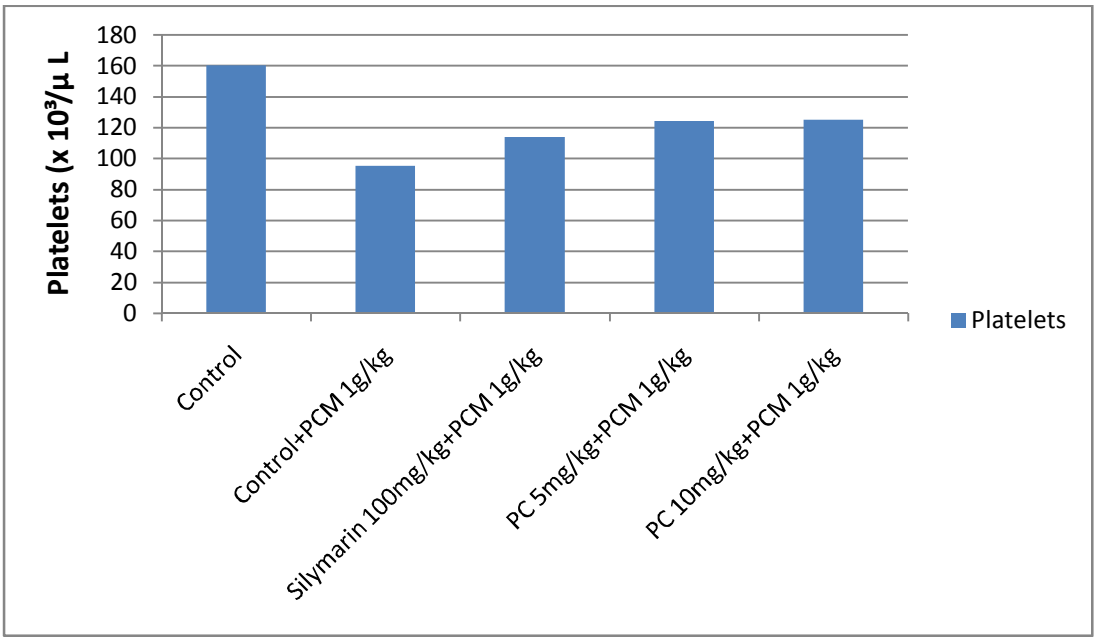


Chart- 27

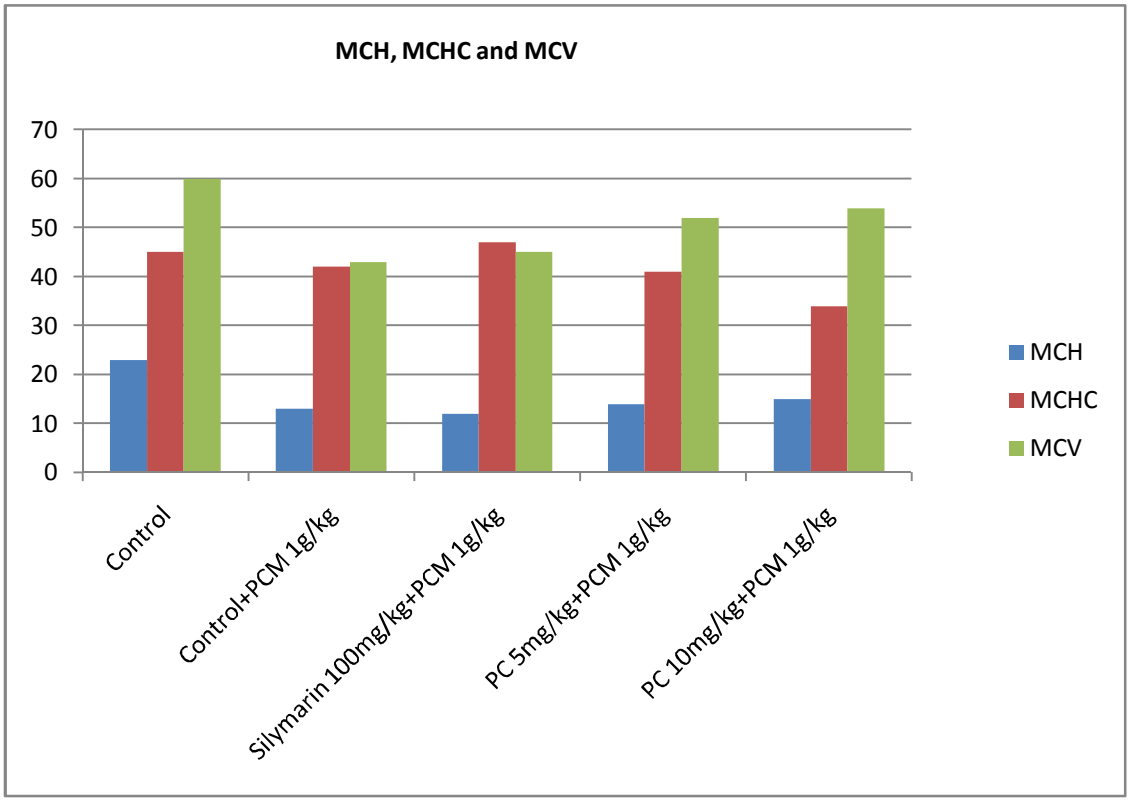


Table 27: Effect of *Panchakkini Chenduram* and Silymarin on serum enzymes (SGPT, SGOT and ALP, Total bilirubin and Total protein on Paracetamol (PCM) induced liver damage in rats

Parameters	**Control**	**Control+ PCM 1g/kg**	**Silymarin 100 mg/kg+ PCM 1g/kg**	**PC 5mg/kg+ PCM 1g/kg**	**PC10mg/kg +PCM 1g/kg**
SGOT (IU/L)	157.3±24.65	300.35±10.73	213.87±6.26*	250.77±4.45 **	160.±4.45 **
SGPT (IU/L)	72.3±14.79	278.05±8.71###	57.67±3.66 **	60.27±4.26**	30.27±4.26
ALP (IU/L)	42.35±1.76	77.67±1.76 ###	41.67±2.70 ***	53.63±1.36 ***	51.63±1.36 ***
Total Bilirubin (mg/dl)	11.03±1.20	15.73±0.45###	6.66±0.85*	3.26±0.40 ***	3.06±0.40 ***
Total Protein (mg/dl)	3.506±0.78	7.76±1.28###	9.83±0.38*	12.67±2.58 *	12.67±2.58 *

PC- *PANCHAKKINI CHENDURAM* ***PCM-PARACETAMOL***

Values are Mean ± SEM; n = 6 animals in each group: #P<0.05, ##P< 0.01, ###P<0.001 is considered significant when compared with group I; *P<0.05, **P< 0.01, ***P<0.001 is considered significant when compared with group II by Tukey multiple comparison test.

Chart- 28

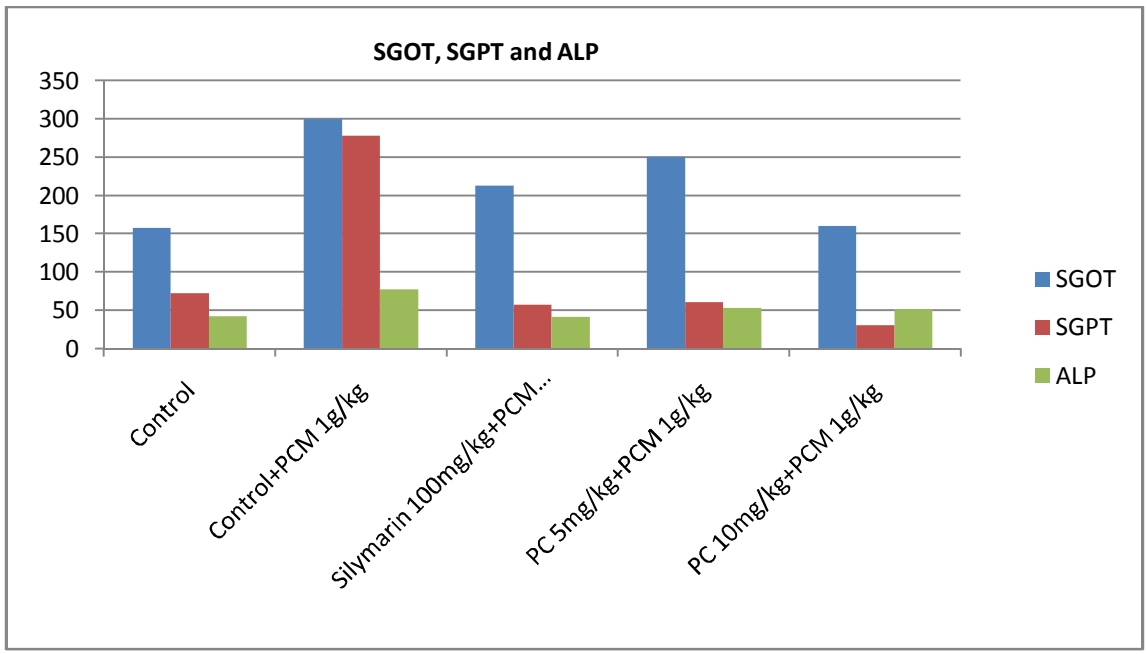


Chart- 29

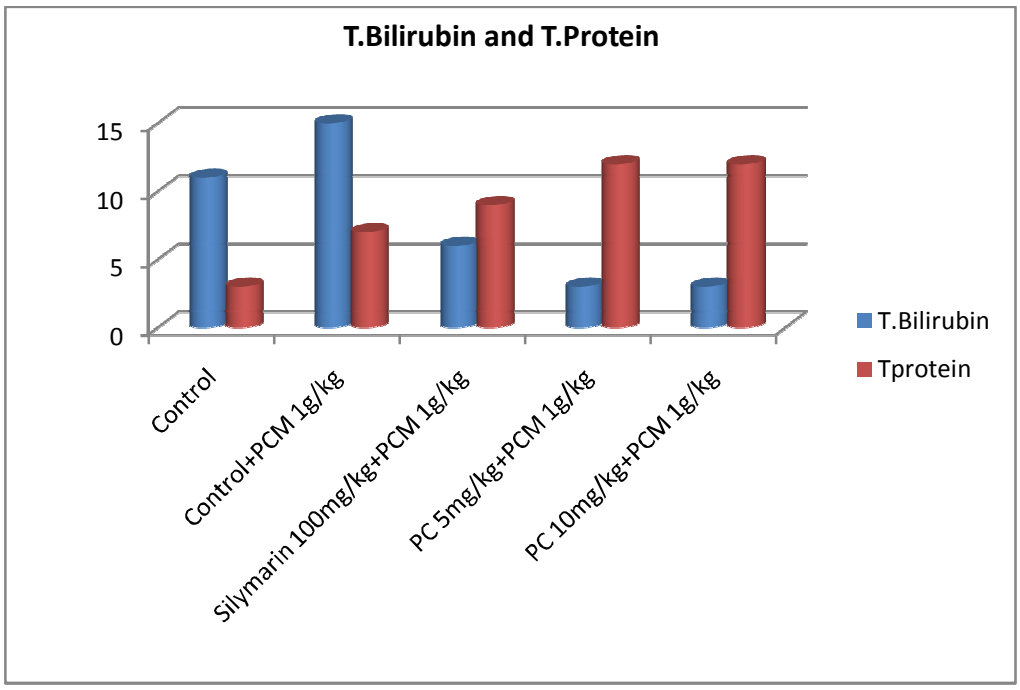


Table 28 : Effect of *Panchakkini Chenduram* and Silymarin on serum Creatinine and Urea, on Paracetamol (PCM) induced liver damage in rats

Parameters	Control	Control+ PCM 1g/kg	Silymarin 100 mg/kg+ PCM 1g/kg	PC 5mg/kg+ PCM 1g/kg	PC10mg/kg+PCM 1g/kg
Creatinine (mg/dl)	0.74±0.05	1.40±0.12	1.33±0.21	1.26±0.25	1.15±0.15
Urea (mg/dl)	17.57±2.18	84.77± 4.80 ***	55.23±4.91 ##	42.04±4.36 ###	36.13±4.45 ###

Values are mean ± SEM. ***P<0.001, **P<0.01, *P<0.05 compared to control rats.### P<0.001, ##P<0.01, #P<0.05 compared with group II by Tukey multiple comparison test.

Chart- 30

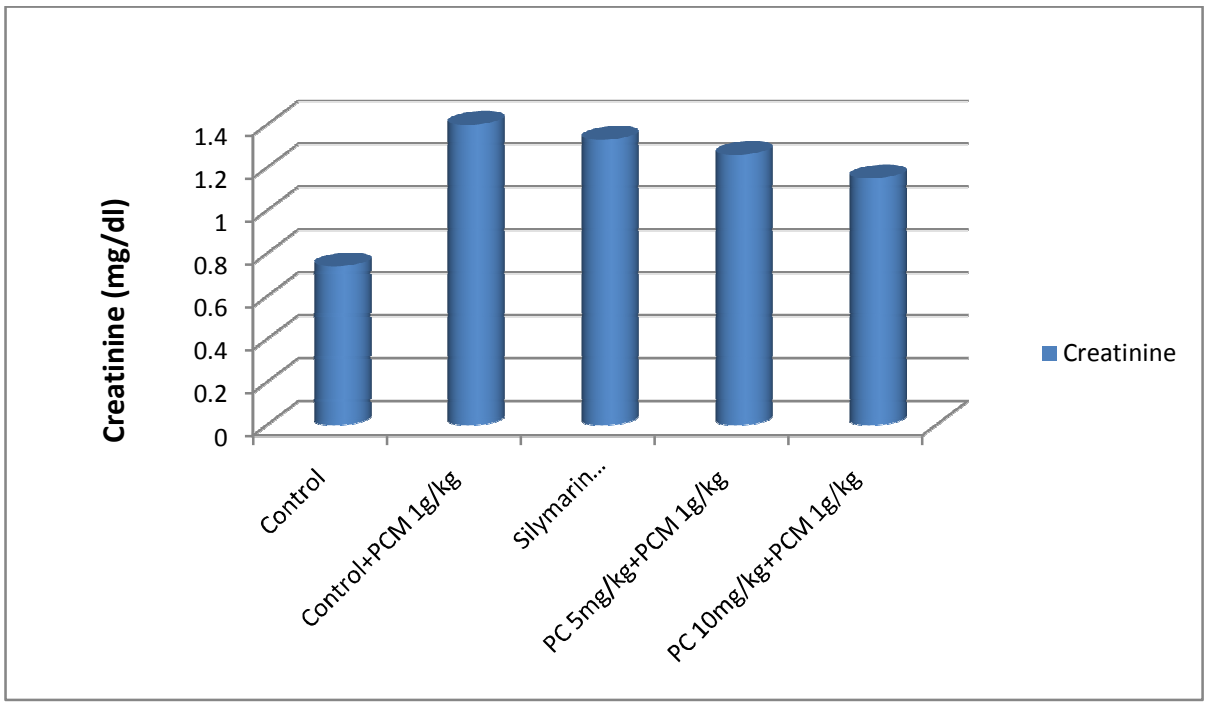
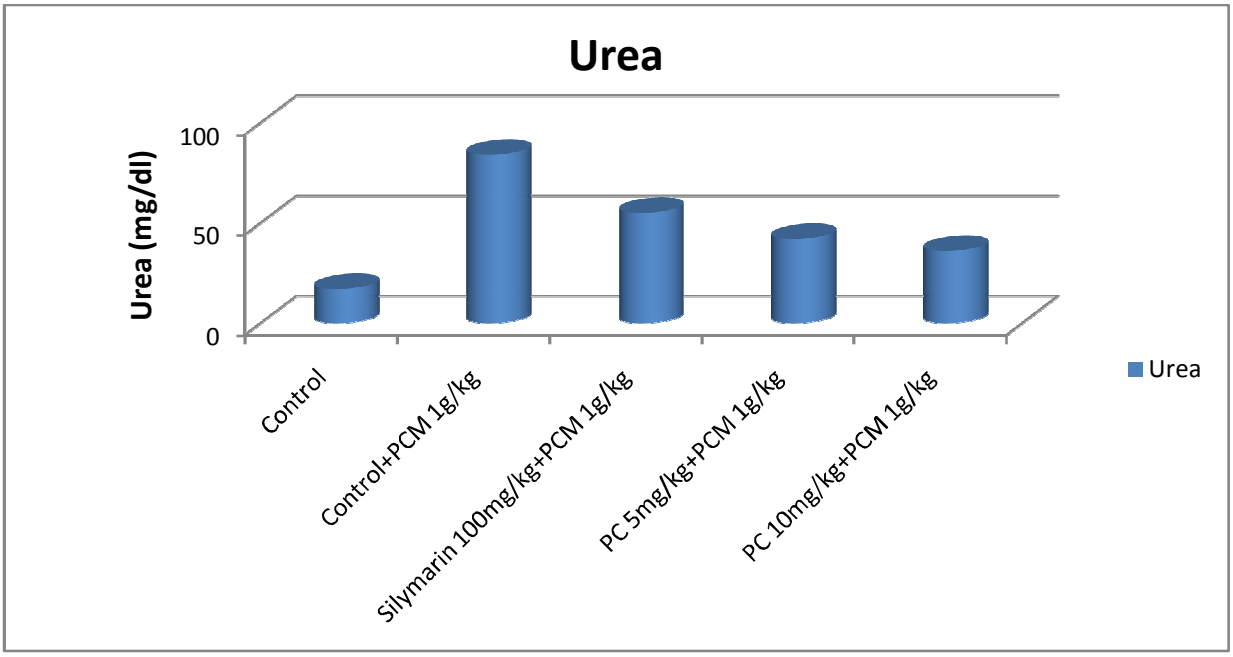
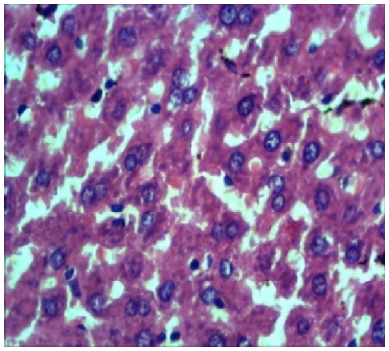


Chart- 31

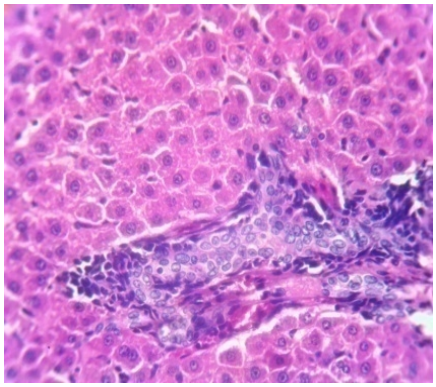

Urea
Urea (mg/dl)
100
50
0
Control
Control+PCM 1g/kg
Silymarin 100mg/kg+PCM 1g/kg
PC 5mg/kg+PCM 1g/kg
PC 10mg/kg+PCM 1g/kg
Urea

Histopathological Report:

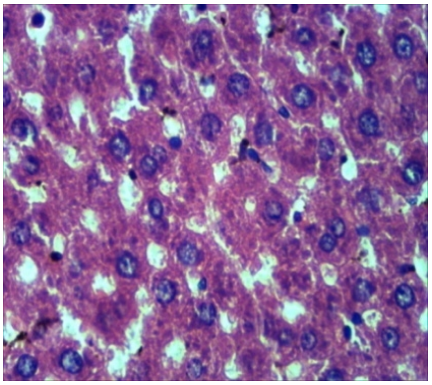
Group 1: Control



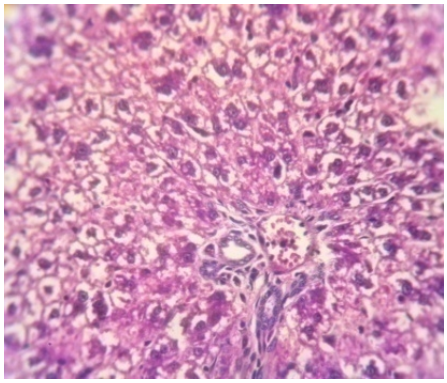
Group2: Control+PCM 1g/kg



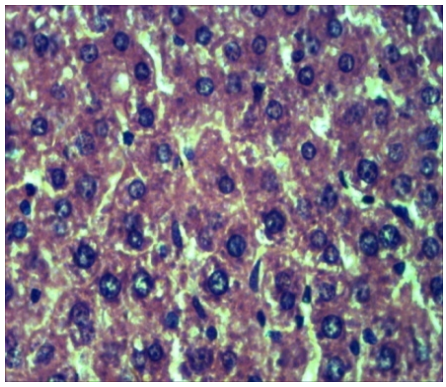
Group 3: Silymarin 100mg/kg + PCM 1g/kg



Group 4 : PC 5mg/kg+PCM 1g/kg



Group 5: PC 10mg/kg + PCM 1g/kg



Interpretation:

Hepatoprotective activity was carried out in Paracetamol induced Wistar albino rats. Paracetamol induced hepatotoxicity. The effects observed were compared with a known hepatoprotective agent, silymarin. In the acute liver damage induced by different hepatotoxins, *Panchakkini Chenduram* (5mg/kg and 10mg/kg, po) significantly reduced the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin. The higher dose of the PCM (1 mg/kg, po) prevented the increase in liver weight when compared to hepatoxin treated control, while the lower dose was ineffective except in the paracetamol induced liver damage. Histological examination of the liver tissues supported the hepatoprotection. It is concluded that the the *Panchakkini Chenduram* possesses good hepatoprotective activity. From the blood samples collected PCV, Hb concentration, MCV, MCH, MCHC, RBC and WBC count were determined.

Conclusion:

In the present study the above parameters analyzed, it may concluded that *Panchakkini chenduram* has significant produced hepatoprotective activity against Paracetamol induced rat.

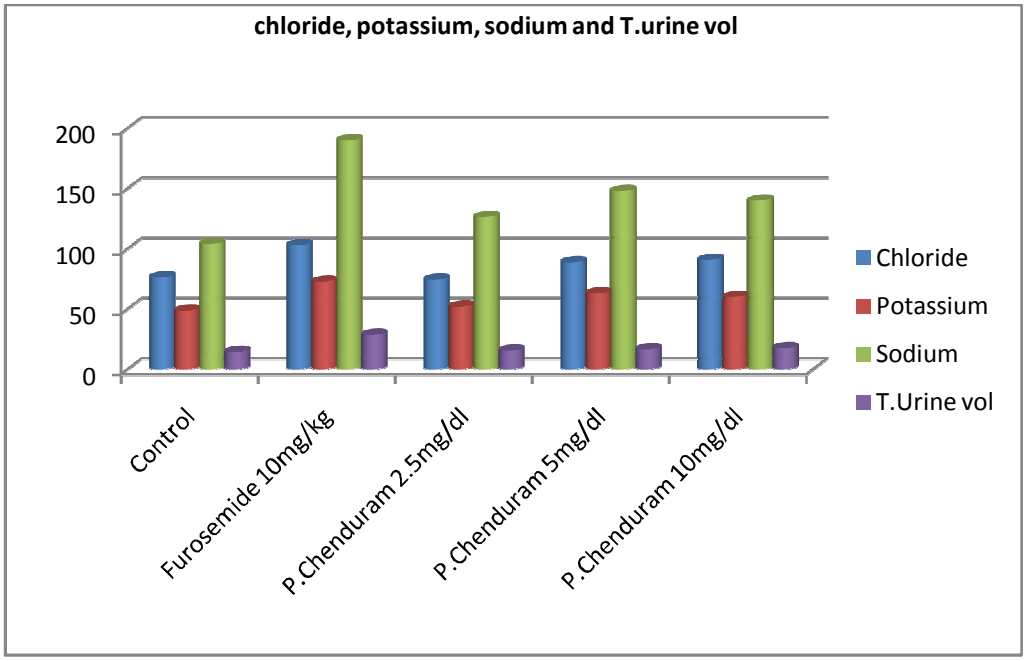
PHARMACOLOGICAL RESULT OF DIURETIC ACTIVITY

Table 29:

Groups	Total Urine Vol (ml/kg/6hrs)	Na+ mmol/L	K+ mmol/L	Cl- mmol/L
Control (10ml/kg)	14.34±0.37	104.0±2.30	48.67±2.02	76.23±2.80
Furosemide (10mg/kg)	28.56±0.83 ***	190.0 ±2.30 ***	72.67±1.45***	102.97±5.51 ***
Panchakkini Chenduram (2.5mg/kg)	15.45 ±0.29 ###	126.3 ±4.05 ###	52.00±2.30###	74.37±2.49 ###
Panchakkini chenduram (5 mg/kg)	16.57 ±0.03 ###	148.0 ±2.30 ###	63.00±1.15 ###	88.77±2.20 ##
Panchakkini Chenduram (10mg/kg)	17.57 ±0.03 ###	140.0 ±2.30 ###	60.00±1.15 ###	90.77±2.20 ##

Values given are as means ± S.E.M, n=6. All the value are compared with the control group(normal saline treated); $p<0.001$

Chart- 32



Interpretation:

Results of study were represented in Table- 29 .They shows the values of urinary volume, Sodium, Potassium and chloride levels.

The control group showed increased urinary sodium potassium and chloride levels compared to standard and drug administrated groups. Significant differences were observed between standard and *Panchakkini Chenduram* treated groups (Group III, IV and V). When compared to lipscitz value of *Panchakkini Chenduram treated* groups, so it is considered as moderate Diuretic.

PHARMACOLOGICAL RESULT OF HEMATINIC ACTIVITY

Table 30 : Effect of *Panchakkini chenduram* on Hb, PCV and RBC content in experimental rats

Parameters	Control	Phenylhydrazine (40mg/kg)	PC(10mg/ kg)	Hematinic syrup(0.68ml /kg/day)
Hb (gm/dl)	13.84 ± 3.63	5.46 ± 2.04#	8.76 ± 2.56*	12.73 ± 1.12*
PCV (%)	46± 3.36	38 ± 2.17#	45 ± 3.22*	42 ± 2.87*
RBC(Million/cu.mm)	5.3±0.12	2.7±0.17#	4.1±0.23*	3.9±0.32*

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group II ($p< 0.001$)

Significantly different from Group I ($p< 0.001$)

Chart- 33

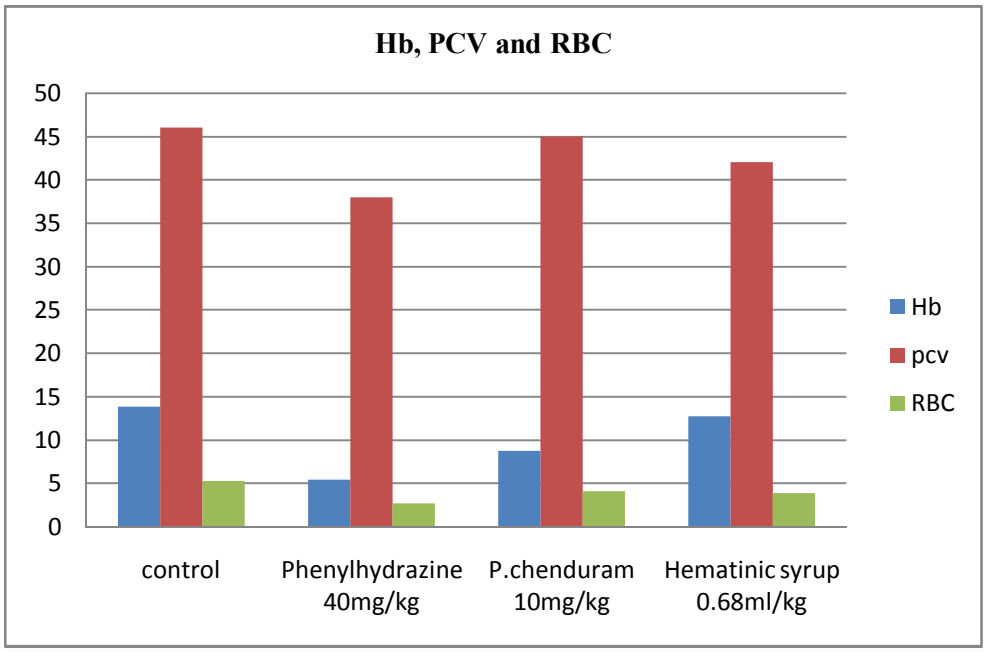


Table 31: Effect of *Panchakkini Chenduram* on WBC and Platelet count in experimental rats

Parameters	Control	Phenylhydrazine (40mg/kg)	PC(10mg/kg)	Hematinic syrup(0.68ml/kg/day)
WBC(cu.mm)	4567±276	2457±254#	3567±234*	3425±275*
Platelet(cu.mm)	350000 ± 3652	120000 ± 9567#	280000 ± 4532*	222000 ± 0.04*

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group II ($p < 0.001$)

\# Significantly different from Group I ($p < 0.001$)

Chart- 34

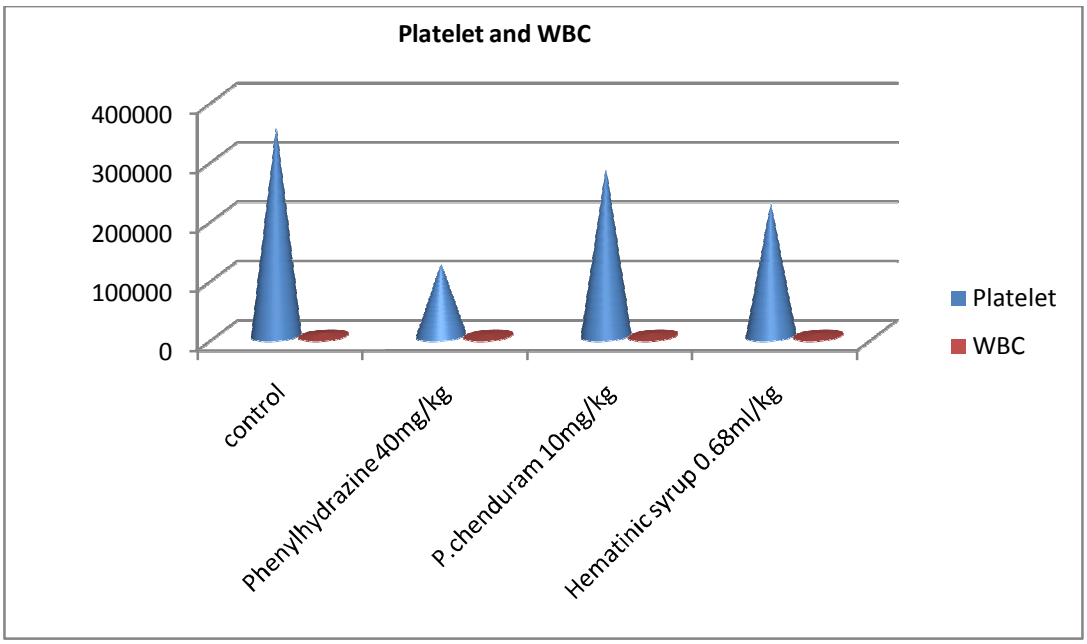


Table 32 :

Parameters	Control	Phenylhydrazine (40mg/kg)	PC(10mg/kg)	Hematinic syrup(0.68ml/kg/day)
SGOT (IU/L)	25.28 ±63.90	91.88 ±22.16#	35.02±13.9 *	40.64± 15.50*

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group II ($p< 0.01$)

Significantly different from Group I ($p< 0.001$)

Chart- 35

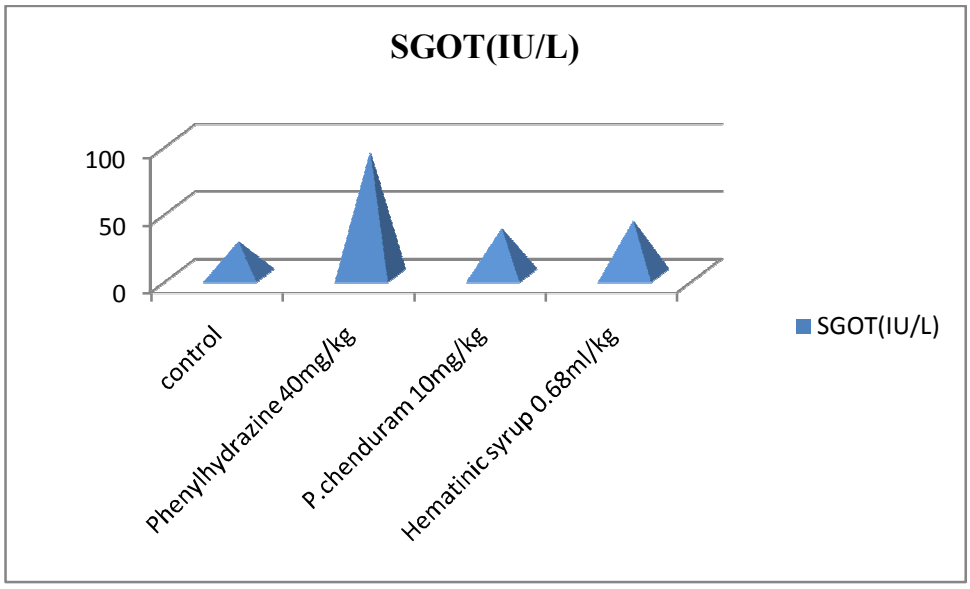


Table 33:Effect of *Panchakkini Chenduram* on MCH, MCHC and MCV count in experimental rats

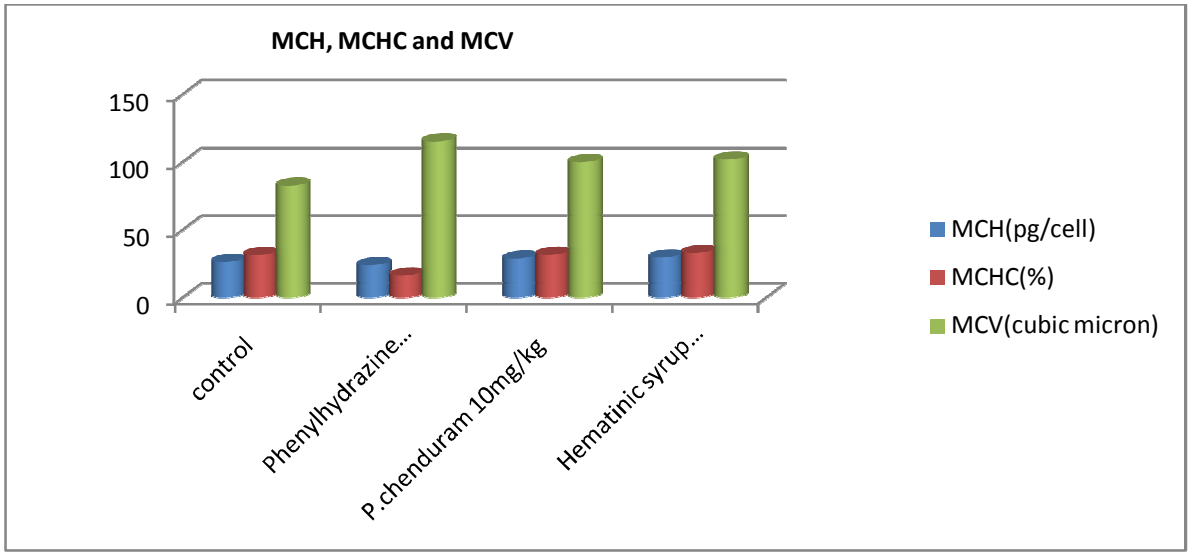
Parameters	Control	Phenylhydrazine (40mg/kg)	PC(10mg/ kg)	Hematinic syrup(0.68ml/ kg/day)
MCH (pg/cell)	26.34± 1.87	23.92 ± 1.60#	28.81 ± 2.24*	29.55 ± 2.22*
MCHC (%)	31.43± 2.55	16.27 ± 1.24#	31.61 ± 2.24*	32.97 ± 2.78*
MCV (Cubic micron)	82.58 ± 5.29	115.23 ±8.27#	100 ± 7*	101.84±7.29*

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group II ($p< 0.01$)

\# Significantly different from Group I ($p< 0.01$)

Chart- 36



Interpretation:

Results of study were represented in Table- 30, 31, 32 and 33 . Anemia is one the numerous ailments calimed to been successfully treated with minerals by traditional medicine practitioners. Many minerals products are used to treat the Anemia. Keeping in view in the present study was evaluated the anti- anemic activity of *Panchakkini Chenduram* Medicine. Phenlihydrazine, a alkylhydrazine was chosen to induce haemolytic anemia. The results of the present study concluded that *Panchakkini Chenduram medicine inhibits anemia induced by phenylhydrazine. The anti- anaemic potential of Panchakkini Chenduram medicine for management of anemia.*

DISCUSSION

8. DISCUSSION

The well known folklore medicine *Panchakkini Chenduram* had been subjected to various studies and it confirms the literature evidences. Literary collections, Physicochemical Studies, Bio-chemical analysis, Toxicity studies, Pharmacological studies were done to prove the Hepatoprotective, Diuretic and Hematinic activities of *Panchakkini Chenduram.* Literary review about the ingredients of *Panchakkini Chenduram* from various text books give hope about its activity. The studies strongly substantiated textual references and as discussed below.

Literary collections:

Literary collections include drug review, which consist Siddha aspect, pharmaceutical and pharmacological review are support for this study.

Drug review:

Drug review about the ingredients of *Panchakkini Chenduram* from various text books was done. *Gunapadam* aspect expressed that the *Panchakkini Chenduram* possess Hepatoprotective, Diuretic and Hematinic property.

Physico chemical analysis:

The pH of the drug was 8.2. It denotes it is slightly alkaline. Hence, in the oral administration of the drug it may it indicates that drug will get ionized in stomach and will be absorbed in intestine and directly sent to portal system.Loss on drying of *Panchakkini Chenduram* at 105°Cis 2.353 %. It indicates the loss of volatile substances along with the water this reveals that drug will not lose much of its volume and volatile substance. It shows that the drug has more stability. Ash value 70.56% it is the residue remaining after incineration that determines the inorganic substances present in the drug. Similarly it can also detect the nature of the material, whether it is adulterate or not. Hence, determination of the ash value provides an idea for judging the identity and purity of the drug

Biochemical analysis:

In bio chemical analysis the results shows that the presence of Silicate, Phosphate, Iron and Magnesium.

Toxicity studies:

In **Acute toxicity study** there is no mortality was observed in animals. Based on OECD 423 the trail drug *Panchakkini Chenduram* is considered as non toxic up to the dose of 2000mg/kg.

The Repeated dose 28 day oral toxicity (OECD - 407)and Repeated dose 90-day oral toxicity study (OECD – 408) of *Panchakkini Chenduram* in Wistar albino rats were studied. The treated animals survived throughout the study period of 28 days and 90 days did not reveal any treatment related major abnormal clinical signs at the test dose levels. The overall percentage of body weight gain in rats treated with the drug was found to be normal indicating that the test animals were in a healthy condition during the 90 days of observation period. The P values of haematological parameters and biochemical parameters of the tested rats were not significant indicating that the drug exerted nil impact on the parameters and they were within the reference range. In histopathological study on UC high dose treated rats. The necropsy studies showed no remarkable changes. This strongly stress the fact of the drug having no toxic effect on the body metabolism.The necropsy studies showed no remarkable changes. So the trial drug *Panchakkini Chenduram* can hopefully used for human trails.

Pharmacological studies:

[

The pharmacological activities like Hepatoprotective, Diuretic, and Hematinic activity of Panchakkini Chendrum shown significant effect.

Hepatoprotective activity

Hepatoprotective activity was carried out in Paracetamol induced Wistar albino rats. Paracetamol induced hepatotoxicity is the generally used screening method for testing the hepatoprotective nature of drugs. The effects observed were compared with a known hepatoprotective agent, silymarin. In the acute liver damage induced by different

hepatotoxins, *Panchakkini Chenduram* (5 and 10mg/kg, po) significantly reduced the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin. The higher dose of the PCM (1 mg/kg, po) prevented the increase in liver weight when compared to hepatoxin treated control, while the lower dose was ineffective except in the paracetamol induced liver damage. Histological examination of the liver tissues supported the hepatoprotection. It is concluded that the the *Panchakkini Chenduram* possesses good hepatoprotective activity. From the blood samples collected PCV, Hb concentration, MCV, MCH, MCHC,RBC and WBC count were determined.

Diuretic activity

The Diuretic activity of *panchakkini Chenduram* is done in Wistar albino rats by Lipschitz test induced method.Wistar rats were divided into five groups, consisting six rats for each group. Group (I) served as normal control (Vehicle) which receivedSaline water (10 ml/kg orally) only. Group II received as furosemide orally dose of (10mg/kg, p.o) Groups (III) to (V) received *Panchakkini Chenduram* respectively at dose of 2.5mg/kg, 5mg/kg and 10 mg/kg respectively. Immediately after the extract treatment, all the rats are hydrated with saline (15 ml/kg) and placed in a metabolic cages. A total volume of urine collected for 6 hours was measured at the end. During this period no food and water were made available to animals. Various parameters like total urine volume and concentration of sodium, potassium and chloride in the urine were measured and estimated respectively.Estimation of urine output is expressed as ml/kg.

Hematinic activity

Diuretic activity was carried out in Phenylhydrazine injection intraperitoneally to induced anemia in Wister albino rats, the rat were divided into 4 groups of 6 rats each. I^{st} group act as control, II^{nd} group received PHZ, III^{rd} group received Panchakkini Chenduram,IV^{th} group received standard as hematinic syrup. On collecting the blood sample, it is concluded that HB, RBC, WBC, Platelet, PCV, MCV, MCHC decreased in group II compared to group I, increased in group III compared to group II, except SGOT. This result supports atleast partially used to anaemia.

ANALYSIS STUDIES

- The **Fourier Transform Infrared Spectroscopy (FTIR)** analysis of *Panchakkini Chenduram* shows Strong intense peak at 466.11 cm-1 may be due to Fe- S stretching indicates the presence of Fe-S group and also shows present of functional groups such as alcohol, carbonyl, amine, amide, sulfide, disulfide, ferrous sulfide.
- **X-Ray Diffraction (XRD)** analysis of the *Panchakkini Chenduram* shows intensity peaks of various places. The peaks were identified as crystalline peaks.
- The **High resonance scanning Electron microscopy (HR-SEM)** analysis shows the shape of *Panchakkini Chenduram*. The micrograph reveals the information on external morphology, texture and orientation of materials making up the sample.
- **Ultraviolet – Visible Spectroscopy** analysis shows the λ max value of the sample *Panchakkini Chenduram* projects intense absorbance at 302.72 nm and 384.33 nm.Also presents of sulfide and Iron oxide.

SUMMARY

9. SUMMARY

- The selection of the drug *Panchakkini Chenduram* from the literature "*Anuboga vaidya navaneetham*" part 1, page no 92, *Hakkim Abdulla shayabu*, for the evaluation of Hepatoprotective, Diuretic and Hematinic activities.
- The test drug was prepared by the given procedure. All the ingredients were identified and authenticated by the experts.
- Review of literature in various categories was carried out Siddha aspect and pharmaceutical review disclosed about the drug and the disease. The pharmacological review was done to establish the methodologies.
- Since the ingredients of *Panchakkini Chenduram* purified according to the classical methods. The purification process of this drug possible to eliminate their toxins and increases its efficacy and the grinding process of this drug helps to reduce the particle size of the drug for its better bio availability.
- The drug was subjected to analysis such as physicochemical, chemical and also instrumental analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug.
- Toxicological study was made according to OECD guidelines comprising acute, sub-acute and sub chronic toxicity study. It screens the safety of the drug which attributes its utility in long time administration.
- Pharmacological study was done. It revealed the Hepatoprotective, Diuretic and Hematinic activities of *Panchakkini Chenduram* in Wistar albino rat model.
- Results and discussion given the necessary justification to prove the potency of the drug.
- Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.
- Thus the mineral formulation *Panchakkini Chenduram* is validated for its safety and efficacy for treating *Liver disesses, Oliguria* and Anaemia it would be a great drug of choice.

CONCLUSION

10. CONCLUSION

From the literature evidence, Physico Chemical Analysis, Chemical Analysis, Toxicological evaluation and pharmacological studies, the drug Panchakkini Chenduram have Hepatoprotective, Diuretic, Hematinic activity it was concluded that the Panchakkini Chenduram can be issued in the management Liver diseases, Oliguria, and Anaemia.

ANNEXURE

Tha Tamil Nadu Dr.M.G.R.Medical University

69,Anna Salai,Guindy,Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr/Mrs~~.....S. Savitha.....

for participating as ~~Resource Person~~ / Delegate in the Seventeenth (XVII) Workshop on

" RESEARCH METHODOLOGY & BIOSTATISTICS "

FOR AYUSH POST GRADUATES & RESEARCHERS

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 15th to 19th June 2015.

Dr.N.KABILAN, M.D.(Siddha)
READER,DEPT.OF SIDDHA

Prof. **Dr.P.ARUMUGAM,** M.D.,
REGISTRAR i/c

Prof. **Dr.D.SHANTHARAM,** M.D., D.Diab.,
VICE - CHANCELLOR

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Jeppiaar Nagar, Rajiv Gandhi Road, Chennai – 600 119, Tamilnadu. India.

CENTRE FOR LABORATORY ANIMAL TECHNOLOGY AND RESEARCH
(CPCSEA Approved)

WORKSHOP ON LAB ANIMAL HANDLING AND RESEARCH
10 – 11th MARCH, 2016

CERTIFICATE

This is to certify that Dr. S. SANTHA of National Institute of Siddha, Chennai has participated in the two-day workshop on "LAB ANIMAL HANDLING AND RESEARCH" organized by the Centre for Laboratory Animal Technology and Research, Sathyabama University, Chennai during 10 – 11th March, 2016.

Director
Dr. Marie Johnson

Director
Dr. Mariazeena Johnson

Vice-Chancellor
Dr. B. Sheela Rani

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Centre For Advanced Research In Indian System Of Medicine (CARISM)

Certificate of Participation

This is to certify that Dr. **S. SAVITHA** of **National Institute of Siddha, Chennai** participated in Ministry of AYUSH supported training programme on **"Characterization Techniques in the Standardization of Ayurvedha & Siddha Herbo-Metallic Preparations"** held during 28 to 30 march 2016.

SHANMUGHA ARTS, SCIENCE, TECHNOLOGY & RESEARCH ACADEMY

P. Brindha
Convener
Prof. **P. Brindha**

Registrar
SASTRA University

CERTIFICATE OF AUTHENTICATION OF MINERAL SAMPLES

Certified that the minerals submitted for identification by Dr.S.Savitha, 2nd year PG Scholar, Department of Gunapadam, National Institute of Siddha, Tambaram sanatorium, Chennai-47 were identified as Magnetic Oxide of Iron,Sulphur,Iron powder, Conch, Ammonium Chloride with below microscopic and macroscopic characteristics based on Rutlys and Danas mineral descriptions.

M. Suresh Gandhi

Dr.M.Suresh Gandhi
Assistant Professor
Department of Geology
Guindy Campus,

Chennai-600025

Date: 28/8/16

Dr. M. SURESH GANDHI, M.Sc.,M.Phill.,Ph.D.,
Assistant Professor
Department of Geology
University of Madras
Chennai-600 025.

C.L.BAID METHA COLLEGE OF PHARMACY

(An ISO 9001-2000 certified institute)

Jyothi Nagar, Old Mahabalipuram Road

Thoraipakkam, Chennai – 600 097

CERTIFICATE

This is to certify that the project entitled, **Pharmacological and Toxicological study of PANCHAKKINI CHENDURAM** in rats submitted in partial fulfilment for the degree of **M.D. (siddha)** was carried out at C.L. Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2015-2016. It has been approved by the **IAEC No: IAEC/XLIX/16/CLBMCP/2016**

C.L. Baid Metha College of Pharmacy CHENNAI-97 Thoraipakkam

(Dr.P.Muralidharan)

IAEC Member Secretary

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